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THE ECONOMIC BOTANY SYMPOSIUM: INTRODUCTION[1]

P. Mick Richardson[2]

> *History celebrates the battlefields whereon we meet our death, but scorns to speak of the plowed fields whereby we thrive; it knows the names of the King's bastards, but cannot tell us the origin of wheat. That is the way of human folly.*—Jean Henri Fabre

The subject of economic botany needs a comprehensive textbook to take us into the next century. Simpson and Conner-Ogorzaly's (1986) *Plants in Our World* is still a useful book, as are the dictionaries by Howes (1974), Mabberley (1987), and Uphof (1968), and numerous more specialized texts on particular geographic areas or groups of plants, but none are a 21st Century replacement for Hill's (1937) *Textbook of Useful Plants* or Schery's (1972) *Plants for Man*. The proposed book could not possibly include all of the uses of plants but it could include the major ones, especially the recent major developments in the use of plant products. It might explain what happens to all the lumber from clearcuts in the Pacific Northwest, why corn rather than lemons is now the source of citric acid, why paper products cannot be indefinitely recycled, why alcohol may be a renewable fuel but has caused the extensive destruction of tropical forests, and would cover subjects ranging from hunter-gatherers to gene-splicing.

In our daily lives we constantly interact with plants and plant products and, although I once participated in teaching a course in economic botany at the University of Illinois, I have always regretted that I never had the chance to take such a class at the undergraduate or graduate level. I have had a lifelong interest in plants, entering the field of biology by way of school classes in horticultural science taken from the age of eleven onwards. At that time I believed that the "Monk's cabbage" (*Brassica oleracea* L.) that grew on the steep cliffs below the nearby abandoned castle/monastery complex at Tynemouth was truly wild and only recently discovered that it is more likely to be an ancient naturalization (Swan, 1993). How-

[1] This and the six articles that follow it are the proceedings of the 40th Annual Systematics Symposium of the Missouri Botanical Garden, *Economic Botany*, which was held 8–9 Oct. 1993 at the Missouri Botanical Garden in St. Louis, Missouri, U.S.A.

The symposium was supported in part by the National Science Foundation under grant number BSR89-18138. I thank Hardy Eshbaugh and Beryl Simpson for help in organizing the symposium, John Myers for his fine illustration for the symposium brochure inspired by an unusual publication by Edgar Anderson, Mr. "Weedy" Wallace for introducing me to the science of plants, and the world's numerous economic botanists for enjoyable conversations and reading material.

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ever, I did know that these cliff-hangers could theoretically be collected and, by judicious selection, used to re-create the various cabbages, kales, broccoli, cauliflower, and brussel sprouts (I was not yet aware of the numerous Oriental relatives) that were grown in the school garden. Similarly, I knew that the prize-winning potatoes of my schooldays had originally come from Peru, but it was only later that I learned that a limited gene pool was the cause of last century's potato crop failures in so many European countries, especially in Ireland, while wheat and other Old World cereals never had similar total crop failures due to the large number of land races then in cultivation. When Steve King told me about the vast number of different kinds of potatoes in cultivation in the Andes, including, remarkably, one grown only for consumption in the fields during the actual potato harvest, my first thought was about how those dreadful famines might have been avoided if only a more diverse range of potatoes had been introduced into Europe.

Although my own research activities have barely touched directly on economic botany, I believe that virtually all studies of plants impinge upon it in one way or another. My work has from time to time brought me to the margins of the field: as a graduate student at Kew in the early 1970s I studied the chemistry of the wild ancestors of spearmint; during my first postdoctoral stint, among other things I looked at phytoalexins in wild and cultivated beets, mainly memorable in that the wild seeds germinated better after an overnight soak in concentrated sulfuric acid; and many years later, as acting manager of the Center for Plant Conservation, I was responsible for filling a postion for a study of the endangered relatives of cultivated plants in the U.S. Finally, two years ago, to my pleasant surprise, I found myself organizing a conference on economic botany.

It was surprisingly easy to choose a range of speakers to cover some of the diversity of areas that fall under the general umbrella of economic botany. Indeed, we covered the spectrum from hunter-gatherers to gene-splicing. Local botanists Gayle Fritz, Walter Lewis, and Robert Fraley fitted the program ideally, covering the subjects of paleoethnobotany, medicinal plants, and commercialization of genetically modified plants, respectively. Jan Salick discussed her work on the interface of ecology and economic botany, and Janis Alcorn brought the audience up to date on the role of tenurial rights in plant conservation. Gordon Cragg informed us of the various stages of preclinical development of pharmaceutical crops. Finally, Charles Heiser gave the evening address on his mentor Edgar Anderson. Six of the seven papers presented at the symposium are published here.

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NEW DATES AND DATA ON EARLY AGRICULTURE: THE LEGACY OF COMPLEX HUNTER-GATHERERS[1]

Gayle J. Fritz[2]

ABSTRACT

The Accelerator Mass Spectrometry (AMS) method of radiocarbon dating has shortened the history of maize agriculture by demonstrating that purported earliest cobs from Mexico, New Mexico, and eastern North America are younger than, and intrusive into, earlier archaeological strata. Models of agricultural origins based on a 5000 B.C. or earlier date for cultigens must be discarded or validated by directly dated specimens. A more recent date (ca. 3000–3500 B.C.) for maize domestication leads to a new focus on settled hunter-gatherers in resource-rich zones. Social complexity in nonagricultural societies elsewhere is becoming more generally appreciated. In the Lower Mississippi Valley, nonagricultural mound builders persisted until 1100 A.D., and in Florida, California, and the Pacific Northwest, stratified hunter-gatherers flourished until Europeans arrived. These examples of sustainable harvesting demonstrate the long-term viability of such systems.

In much of the Western Hemisphere, we can no longer state that agriculture began as long ago as outlined in many currently used textbooks. Examples include the sequence for maize domestication in Mexico and the spread of maize to the Greater Southwest and the region that is now the eastern United States. The standard scenarios, even though they have been undermined by new dates and new data, have a strong hold on professionals and informed members of the general public. Maybe people think it will be only a few years until new discoveries are made that push back the chronology to its prior position if not farther. Maybe we cling to the old models out of respect for our pioneering mentors. Whatever forces are operating, it is time to go public with the new, younger dates and to accept the damage to cherished scenarios.

I begin this paper by evaluating the impact of the Accelerator Mass Spectrometer (AMS) method of radiocarbon dating on the study of agricultural evolution in the New World. I then turn to evidence generated by another technological innovation—archaeological flotation—and summarize the unexpected results of archaeobotanical studies in the Midwestern United States and the Lower Mississippi Valley. Nonagricultural societies persisted longer than previously believed in both Mexico and the Lower Mississippi Valley. In Louisiana, dense populations of sedentary and socially complex mound builders preceded the adoption of maize. This leads to a discussion of complex fisher-gatherer-hunters in general, because groups in the Lower Mississippi Valley seem similar in many ways to other sedentary, nonagricultural peoples, including the Natufians and Epipaleolithic villagers of the ancient Near East, the Calusa of Florida, and native American groups in California and the coastal Pacific Northwest. The western North American groups practiced what seems to qualify as sustainable harvesting quite successfully for millennia, until the European incursion, supporting population densities exceeding those of all farming societies north of Mesoamerica.

I believe we can apply this knowledge to discussions of modern resource management and ecological imbalance, and I conclude this paper by emphasizing the viability of sustainable harvesting when it is put in long-term perspective.

NEW DATES ON "EARLY" MAIZE

THE AMS RADIOCARBON METHOD AND ITS IMPACT

Radiocarbon dating has been an essential tool for archaeologists since Willard Libby offered it to scientists in 1949 (Taylor, 1987). Many techno-

[1] I am grateful to Mick Richardson for inviting me to participate in this symposium and to Ksenija Borojevic, David Browman, Fiona Marshall, Patty Jo Watson, and Henry Wright for enlightening and encouraging me while working on these issues. I also thank Dean Martin Israel and the Faculty of Arts and Sciences of Washington University in St. Louis for granting release time from teaching, without which this research would have been impossible.

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TABLE 1. AMS radiocarbon dates on maize cobs from Coxcatlán and San Marcos caves, Tehuacán Valley, Mexico (from Long et al., 1989).

C-14 age (years B.P.)	Uncalibrated midpoint	Calibrated range (1-sigma)
450 ± 40	A.D. 1500	A.D. 1400–1460
1560 ± 50	A.D. 390	A.D. 440–620
1860 ± 45	A.D. 90	A.D. 80–220
1900 ± 60	A.D. 50	A.D. 20–200
3740 ± 60	1790 B.C.	2280–2040 B.C.
4040 ± 100	2090 B.C.	2580–2500 B.C.
4090 ± 50	2140 B.C.	2870–2580 B.C.
4150 ± 50	2200 B.C.	2880–2660 B.C.
4600 ± 60	2650 B.C.	3380–3360 B.C.
4680 ± 50	2730 B.C.	3500–3380 B.C.
4700 ± 60	2750 B.C.	3500–3380 B.C.
4700 ± 110	2750 B.C.	3640–3360 B.C.

logical improvements have been made during the last 45 years. The innovation that concerns us most is the AMS method, which enables very small samples to be assayed. Laboratories request 5–10 grams of organic material for a standard age determination, but that much charcoal or other organic matter in tight archaeological context is difficult to find in many sites. Radiocarbon dating is a destructive method: the object submitted is converted to gas or liquid benzene solution. For many decades, therefore, valuable samples such as early corn cobs could not be sacrificed and had to be considered the same age as associated wood charcoal or other organic material.

The AMS method, however, requires only a few milligrams of organic matter, so that individual seeds or very small pieces of larger items can be directly dated (Hedges & Gowlett, 1986). The reason that, in some regions, the earliest evidence for certain crops has become younger is that many specimens recently subjected to AMS radiocarbon dating turned out to be intrusive into the older strata in which they were encountered. This is especially common in caves and rockshelters, where slabs fall from the roof, animals burrow, and people dig new pits during subsequent occupational episodes. It also happens at open-air sites, however. A widely known example is the barley (*Hordeum vulgare* L.) from Wadi Kubbaniya, Egypt, dated by stratigraphic association to 17,000 b.p., but determined by direct AMS radiocarbon dating to be only 5000 years old (Wendorf et al., 1979; Wendorf et al., 1984). The early dates were big news. The later ones were made available to the scientific community, but were not, to my knowledge, picked up by the press.

REVISING THE CHRONOLOGY FOR MAIZE DOMESTICATION IN MESOAMERICA

The most sacred scheme for agricultural origins undermined by AMS radiocarbon dating is the sequence in the Tehuacán Valley of Puebla, Mexico. Maize (*Zea mays* L. subsp. *mays*) cobs from levels in which some material was as old as 5000 B.C. form the backbone of competing theories for the domestication of this, the most important food plant of millions of native Americans for millennia (Benz & Iltis, 1990; Doebley, 1990; Galinat, 1985; Mangelsdorf, 1974). None of the original radiocarbon samples from these sites included actual maize material, because the specimens were too small and much too valuable to be sacrificed.

Recently, AMS radiocarbon dates were acquired using tiny pieces of 12 maize specimens from three of the Tehuacán Valley rockshelters: Cueva San Marcos, Cueva Coxcatlán, and Cueva Purrón (Long et al., 1989). Richard MacNeish, the original excavator, selected the 12 samples that he considered to have the "best proveniences and relationships to well-dated levels" (Long et al., 1989: 1036). Eleven samples came from levels assigned to the Coxcatlán Phase (3500–5000 B.C.) and one from the Abejas Phase (2500–3500 B.C.). To make a long story short, all of the cobs thought to be 5500–7000 years old (3500–5000 B.C.) were determined to be significantly more recent (Table 1). The oldest midpoint date (before dendrocalibration) is 2750 B.C.; the youngest specimen turned out to be only 500 years old (450 b.p. ± 40: A.D. 1500). Three other cobs were assayed as falling within the past 2000 years: A.D. 50, A.D. 90, and A.D. 390. The rest fall within the second and third millennia B.C., not the fourth, fifth, or sixth millennia as published in hundreds of books and articles (e.g., Fagan, 1994: 302; Fiedel, 1992: 181; Jennings, 1989: 258).

The authors of the report for the journal *Radiocarbon* (Long et al., 1989) soften the blow of these dates by using the one-sigma calendric date ranges after calibration. This pushes four of the cobs back into the fourth millennium B.C., and makes it possible to envision the earliest specimen as going back to 3640 B.C. If the original dates, those based on associated charcoal, were viewed as dendrocalibrated, one-sigma ranges, however, they too would be pushed back more than 1000 years, so the discrepancy remains a major one.

The consequences for both archaeologists and botanists go beyond teaching us to have greater respect for post-depositional processes that result in the mixing up of sediments and artifacts of

various ages. Maize may not have been domesticated until approximately 3500 B.C. The search for earlier evidence is ongoing, but if domestication had occurred as early as once believed, why would the earliest dated maize in the Tehuacán rockshelters, now accepted as fully domesticated (Benz & Iltis, 1990), be so primitive-looking that Mangelsdorf et al. (1967b) argued that it was wild and others described it as transitional between wild teosinte and domesticated maize (cf. Benz & Iltis, 1990)?

I must assume for now that all of the undated cultigens from Tehuacán (Mangelsdorf et al., 1967b; Smith, 1967), Tamaulipas (MacNeish, 1958; Whitaker et al., 1957), and Oaxaca (Flannery, 1986; Whitaker & Cutler, 1971) are intrusive into the earlier strata in which excavators found them. This means we have no documentation for 9000-year-old *Lagenaria siceraria* (Mol.) Standley or *Cucurbita pepo* L.—nor even 7000-year-old cucurbits—no Mesoamerican agriculture until after 4000 B.C. Early food production in Mexico, then, would have begun thousands of years later than in the Old World, where cereal domestication is well documented by 8000 B.C. (Bar Yosef & Belfer-Cohen, 1992). It becomes necessary to re-evaluate models in the New World that rely on early Holocene rather than middle Holocene climatic variables, to reconsider population growth as a condition preceding plant domestication, and to drastically shorten the time interval between incipient agriculture and maize-based village societies.

PREAGRICULTURAL SEDENTISM IN THE BASIN OF MEXICO

Archaeologists working in the southern part of the Basin of Mexico suggested some years ago that sedentary preagricultural societies developed there as early as 6000 B.C. Abundant plant remains were found at the Zohapilco site, including grains of teosinte, which was then classified as *Zea mexicana* (Schrader) Kuntze. Mammals, turtles, fish, and lake birds were evidently easily and frequently procured. Early agricultural strategies appear here after 3000 B.C., with crops including *Amaranthus hypochondriacus* L., *Physalis* sp., and *Capsicum* sp. No charred maize was reported, but *Zea* pollen was larger and three times more abundant in strata postdating 3000 B.C. than it had been earlier (Niederberger, 1979: 137).

Christine Niederberger reported these finds in the journal *Science* in 1979, stressing the difference between the developmental sequence in the Basin of Mexico and the ones reported from Tehuacán and Oaxaca. The Tehuacán model—taught as gospel to two generations of archaeologists and still in 1993 popular among textbook authors and professors—has key plant species domesticated earlier, but sedentary settlements not occurring until after 2000 B.C. Niederberger (1979: 140) wrote:

> . . . in the lacustrine environment of the temperate highlands . . . and probably in some estuarine zones of coastal Middle America as well, it would seem that this [Tehuacán] scheme of socioeconomic evolution in Post-Pleistocene times, from semi-nomadic mainly gathering groups to sedentary fully agrarian societies, is not applicable. Artifactual and nonartifactual evidence from the lacustrine shores of the Chalco Basin already suggest the existence of fully sedentary human communities in this region from at least the sixth millennium B.C.

Consequences of an early sedentary economy include preagricultural territorialism, a higher rate of population growth, increased manipulation of useful plants, and "a more integrated sociopolitical organization" (Niederberger, 1979: 141).

In addition to making the dominant model for agricultural origins in the New World much less plausible, a shifting forward in time of the sequence of maize domestication in Mexico also shortens the interval between the earliest Mesoamerican farmers and their counterparts in the Greater Southwest and in eastern North America. It is to people living north-of-the-border that I turn next.

I ignore arguments that maize and other cultigens were present in South America much earlier than the directly dated Tehuacán maize cobs (Pearsall, 1992). Unfortunately, all available South American evidence is either in the form of rockshelter plant remains dated only by stratigraphic association and quite possibly intrusive, as at Tehuacán, or pollen, or phytolith evidence. No direct dates on cultigens are currently available and until they are produced, I must take the conservative view that an early South American sequence is unsubstantiated.

EARLY AGRICULTURE IN THE U.S. SOUTHWEST

Some of the earliest radiocarbon samples to be processed in Libby's lab at Chicago came from Bat Cave in western New Mexico (Arnold & Libby, 1950; Dick, 1965; Libby, 1951; Mangelsdorf et al., 1967a). Dates on wood charcoal, together with morphological characteristics of the cobs in comparison with maize from Tehuacán, constituted the basis for the long-held tenet that maize agriculture had diffused into the Southwest from Mesoamerica

before 2000 B.C. (Haury, 1962; Jennings, 1974; Woodbury & Zubrow, 1979). The original Bat Cave cobs were treated with a lacquer that contaminated them for radiocarbon dating (Wills, 1988: 126), but archaeologists from the University of Michigan returned to Bat Cave in the 1980s for further excavations. Direct AMS radiocarbon dates on newly excavated maize and squash established the presence of both cultigens in the Southwest by approximately 1000 B.C. rather than 2000–2500 B.C. Directly dated plant remains from at least five other Southwestern sites are nearly as old as or slightly older than their counterparts at Bat Cave (Wills, 1992: 154). Considering one-sigma date ranges after calibration, Wills (1992: 153) suggested that 1200–1500 B.C. is a convenient time estimate for the earliest farming in this region.

Most Southwestern archaeologists think native Archaic hunter-gatherers adopted cultigens that had been passed from group to group across northwest Mexico (Minnis, 1985, 1992; Willis, 1988, 1992), but some see evidence for migration of farming families into the region (Berry, 1985; Huckell, 1990). Those who doubt actual migration of people disagree about the initial impact of cultigens and the degree of commitment to early agricultural pursuits.

Recent excavations and paleoethnobotanical analyses in the Tucson Basin of Arizona have resulted in the hypothesis that preagricultural societies with access to dependable water sources in high diversity zones of the Sonoran Desert were largely sedentary (Fish et al., 1990). Settlement patterning and architecture during the early agricultural first millennium B.C. seem little different from those of the preceding period. Mesquite beans (*Prosopis* spp.) were probably staple foods before and after the introduction of maize, along with cactus fruits and seedy annuals. This pattern is not altered until many centuries after the development of Hohokam culture, with its fine pottery and irrigation systems. The archaeologists who directed this research proposed (Fish et al., 1990: 77):

> Seasonally mobile bands cannot be assumed to represent the only possible precursor groups to early cultivators in arid and semi-arid portions of North America. . . . Where . . . environmental constellations were optimal, residential stability could have been possible to a degree that cultivation did not entail substantial alteration of seasonal schedules.

So far, then, the earliest evidence for maize agriculture has been pushed forward in time toward the present by approximately 2000 years in Mesoamerica and nearly 1000 years in the Southwest. In both of these regions, sedentary groups exploiting rich biological zones may have been the first farmers, not the nomadic foragers of classic scenarios. Stratigraphic associations have proven untrustworthy, especially in rockshelter sites. I emphasize this information because the old schemes—especially the "7000-year-old" maize from Tehuacán and belief in even older cucurbits from Oaxaca—are so widespread and so slow to be edited out of textbooks. The way things stand right now is also temporary, of course. New evidence will turn up, and some of it might be very old. It must, however, be directly dated.

Eastern North America: Independent Center of Plant Domestication

Eastern North American prehistory has also been altered by AMS radiocarbon dating, and here, as elsewhere, the timing of agricultural origins has been affected. In spite of little direct evidence in the form of actual plant remains, the 1000 B.C. boundary between the Late Archaic and Early Woodland periods used to be a convenient date for the beginnings of maize agriculture in the East (Fagan, 1974; Willey, 1966). Most societies in the region, however, seemed unaffected by the transition to maize farming that was allegedly occurring in some river valleys during the first millennium B.C. Moreover, the importance of introduced cultigens in the diets of people who were ostensibly growing them was hotly debated. A major complication in this region since the 1920s (Gilmore, 1931; Harrington, 1924; Jones, 1936; Linton, 1924) has been the ubiquity of native seed types, some present in earlier contexts than maize and having a "cultivated" look about them.

Direct dating of maize fragments invalidated specimens thought to go back as far as 800 B.C., but it has validated tiny fragments from several sites, giving us a current estimate of 100 B.C. for introduction of corn into the East (Conard et al., 1984; Chapman & Crites, 1987; Fritz, 1993). Native seed plants were being domesticated 2000 years earlier (Table 2), as demonstrated by direct dates on larger-than-wild sunflower (*Helianthus annuus* var. *macrocarpus* (D.C.) Cockerell) and sumpweed (*Iva annua* var. *macrocarpa* (Blake) Jackson) achenes (Asch & Asch, 1985; Conard et al., 1984; Crites, 1993). *Chenopodium berlandieri* Moq. subsp. *jonesianum* Smith was domesticated before 1000 B.C. (Smith & Cowan, 1987), with local sequences supporting the inference that this crop was part of an indigenous, temperate gardening complex and not an import from Mesoamerica (Fritz, 1990; Fritz & Smith, 1988; Smith,

1989). *Polygonum erectum* L., *Phalaris caroliniana* Walter, and *Hordeum pusillum* Nuttall were incorporated into eastern farming systems by the early first millennium A.D., and, although these fruits lack striking morphological characters to distinguish them from wild counterparts, their frequencies, contexts, and associations mark them as part of the early seed cropping complex (Fritz, 1990, 1993; Johannessen, 1988; Watson, 1989).

The presence of *Cucurbita* cf. *pepo* rind in 7000-year-old deposits in Illinois (these were directly AMS radiocarbon-dated and reported by Conard et al., 1984) stole some of the thunder from the early native cultigens because squash was viewed as a tropical cultigen and, as such, would pre-date and possibly serve as a source of inspiration for indigenous seed cultivation north-of-the-border. We now have an alternative scenario to that involving diffusion northward of early domesticated pepo squashes. Two subspecies of *C. pepo* have been recognized on the basis of allozyme frequencies (Decker, 1988), and these groups are validated by chloroplast DNA research (Wilson et al., 1992). One group, designated *C. pepo* subsp. *ovifera* (L.) Decker (Decker, 1988), includes the squashes and gourds typically grown by prehistoric eastern North American Indians and may well have been domesticated in this region (Heiser, 1989; Smith et al., 1992). The 7000-year-old rind from Illinois probably reflects harvesting of wild or ruderal native gourds. By 2300 B.C., however, *Cucurbita pepo* seeds from the Phillips Spring site in western Missouri had increased slightly in size (King, 1985), and by 1100 B.C. pepo seeds from the Marble Bluff site in northwestern Arkansas were clearly from domesticates (Fritz, 1986). Dates for early domesticated *C. pepo* subsp. *ovifera* fall within the range acquired for domesticated sunflower, sumpweed, and chenopod (2500–1000 B.C.), so there is no longer a necessity to import any tropical cultigen from Mesoamerica as a stimulus or model for eastern North American seed cultivation.

Eastern North America now holds the status of an independent center of plant domestication (Smith, 1989). Cultigens were present here slightly before they have been detected in the U.S. Southwest, and the Southwestern crops diffused from Mesoamerica, whereas the eastern ones were domesticated locally. Some Early Woodland groups were more sedentary and arguably more dependent upon crops during the first millennium B.C. than were their Southwestern contemporaries, in spite of the fact that maize was not being grown yet in the East.

TABLE 2. Chronology of prehistoric food production in the eastern U.S.

Post-A.D. 1200	Starchy seeds decline; maize, beans, squash dominate
A.D. 900–1200	Maize established as staple
A.D. 1000	Common bean (*Phaseolus vulgaris* L.)
	Grain Amaranth (*Amaranthus hypochondriacus* L.)
	Cushaw squash (*Cucurbita argyrosperma* L. H. Bailey subsp. *argyrosperma*)
A.D. 500	Pale-fruited chenopod (probably *Chenopodium berlandieri* Moq. subsp. *jonesianum* Smith)
A.D. 100	Tobacco (*Nicotiana ?rustica* L.)
100 B.C.	Earliest known maize (*Zea mays* L. subsp. *mays*)
300 B.C.–A.D. 1000	Heavy starchy seed use: *Chenopodium berlandieri* subsp. *jonesianum*, *Phalaris caroliniana* Walter, *Polygonum erectum* L., *Hordeum pusillum* Nuttall
1500 B.C.	Thin-testa chenopod (*Chenopodium berlandieri* subsp. *jonesianum*)
2300 B.C.	Domesticated pepo gourd/squash (*Cucurbita pepo* subsp. *ovifera* (L.) Decker var. *ovifera*)
2300 B.C.	Domesticated sunflower (*Helianthus annuus* var. *macrocarpus* (D.C.) Cockerell) and sumpweed (*Iva annua* var. *macrocarpa* (Blake) Jackson)
5000 B.C.	Use of wild(?) gourds (*Cucurbita pepo* L. subsp. *ovifera* and *Lagenaria siceraria* (Mol.) Standley)

After maize was introduced, at about the time of Christ, it remained a minor crop for between 600 and 1200 years, depending upon the subregion. In some places, including the Greater St. Louis area, intensification of maize agriculture was accompanied by intensification of the native seed crops (Lopinot, 1991). The farmers at Cahokia, Illinois, and surrounding sites began growing more chenopod, maygrass, and erect knotweed between A.D. 800 and 1100, along with greater quantities of maize. It was not until the last few centuries before European contact that the native seeds declined and the trinity of corn, beans, and squash dominated most fields in what is now the eastern United States.

As in both Mesoamerica and the U.S. Southwest, the earliest maize in eastern North America is not as early as once believed. In some parts of the East, however, the chronology for pre-maize ag-

TABLE 3. Comparison of agricultural chronologies in Mexico, the Southwest, and eastern North America.

	Mexico	Greater Southwest	Eastern U.S.
A.D. 1600			
			Maize farming villages
A.D. 1000			
		Maize farming villages	Earliest dated maize
0			
			Pre-maize farming societies
1000 B.C.	Maize farming villages	Earliest dated maize and squash	Earliest native cultigens
2000 B.C.			
3000 B..C	Earliest dated maize		
4000 B.C.			
5000 B.C.	Undated maize		
6000 B.C.			
7000 B.C.	Undated cucurbits		

riculture has been pushed back to make it the earliest food production north of Mexico. If we disregard for now all undated and arguably intrusive cultigens in early rockshelter strata in Mexico, and hence use 3500 B.C. as the approximate age of initial plant domestication in Mesoamerica, then the first native eastern North American farmers appear downright precocious (Table 3).

This may, of course, be an artifact of the current state of research. Eastern North American archaeologists have conducted more intensive flotation for recovery of small seeds and other charred plant materials, and it has been easier for us to acquire funds and permission to date relevant samples than has been the case elsewhere. Studies into the domestication of grain amaranths and chenopods are underway in Mexico (McClung de Tapía, 1992), and these hold promise for expanding the Mesoamerican sequence. Still, I repeat, we cannot cling to notions from the past that have been severely undermined, particularly the notion that good evidence exists for Mesoamerican agriculture at 5000 B.C. or earlier.

PERSISTENCE OF WILD RESOURCE HARVESTING IN THE LOWER MISSISSIPPI VALLEY

My own primary research territory for the past four years has been east central Louisiana, particularly Tensas Parish, which, along with the lower Yazoo River basin in Mississippi, is the heartland of Coles Creek Culture. The Early Coles Creek period begins at A.D. 700 (Table 4), with well-made ceramics and regional mound centers distributed across the ridge-and-swale topography of the bottomland zone between the Tensas and Mississippi rivers. Coles Creek mounds served primarily as platforms for specialized structures or activities, probably of a ritual nature. They have been interpreted as early signatures of hierarchically ranked societies of the sort anthropologists call chiefdoms (Fritz & Kidder, 1993; Kidder, 1992; Steponaitis, 1986). These were by no means the earliest mound builders in the Lower Mississippi Valley, but Coles Creek sites are relatively early for platform mounds oriented around central plazas, which is the typical pattern for complex Mississippian cultures. "Mississippian" is the label for the agricultural chiefdoms that arose in eastern North America just before A.D. 1000 and persisted until the European invasion. Because maize was the foundation for Mississippian subsistence elsewhere, it has long been assumed that, from the beginning, Coles Creek people practiced maize agriculture. When the importance of pre-maize farming in the Midwest became recognized, attention turned to the Lower Mississippi Valley as a possible cradle for the domestication of native seed crops (Cowan, 1985: 242; Ford, 1985: 349).

My colleague T. R. Kidder and I implemented flotation recovery at the Osceola mound site in 1989, speculating that native seed cropping would be more important than maize production during the early Coles Creek period (before approximately

TABLE 4. Subsistence change in the Lower Mississippi Valley.

Culture	Time	Major plant foods	Other plant foods
Natchez	A.D. 1730 – A.D. 1600	Maize, squash, pumpkins, beans, fleshy fruits	Seeds of native annuals; Old World crops
Plaquemine	A.D. 1500 – A.D. 1200	Maize, acorns, fleshy fruits, tubers (?)	Pecans, hickory nuts, squash/gourd, seeds
Late Coles Creek		Acorns, fleshy fruits, tubers (?)	Maize, pecans, seeds
	A.D. 900		
Early Coles Creek	A.D. 700	Acorns, fleshy fruits, tubers (?)	Seeds, pecans
Troyville	A.D. 400	Acorns, fleshy fruits	Seeds, pecans

A.D. 900). What we found, however, were indications of intensified management of wild resources—primarily acorns (*Quercus* spp.), fruits (*Diospyros virginiana* L., *Vitis* spp., *Rubus* spp., *Sabal minor* (Jacq.) Pers.), fish, turtles, and deer—along with evidence for tubers and some harvesting of seeds, but not of domesticated native crops (Table 4). Squash rind is present, presumably *C. pepo* subsp. *ovifera*, but the tiny pieces cannot be distinguished as domesticated rather than wild. My guess is that they were crops, but that their cultivation was casual and their food value secondary to technological use as vessels and possibly net floats.

Test excavations at a slightly earlier site near Osceola, combined with subsistence remains from much earlier components reported by other archaeologists, dating as far back as 1000 B.C., show that the Lower Mississippi Valley was not the early agricultural center once believed (Jackson, 1989; Kidder & Fritz, 1993). It was instead a rich region where hunter-gatherers developed the technology for sustaining relatively dense, sedentary populations controlled and integrated by complex social mechanisms. By Coles Creek times, people had probably relinquished the strong ethic of food sharing called "balanced reciprocity." They were less committed to egalitarian principles than before and concerned to some degree with the relative status of their kin groups. Clans or lineages may have had restricted ownership of fishing and acorn harvesting territories. Kinship groups may have been responsible for building and maintaining fish weirs and for thinning and burning orchard-like oak groves.

I think the mound centers were places where communities came together for ritually regulated occasions. Feasts held at these times would display the wealth of the hosts and conceivably also the guests, if they were expected to bring food or other goods. A competitive spirit to the festivities would stimulate surplus production. Distribution of surpluses would even out imbalances caused by localized fluctuations and, at the same time, create obligations that indebted groups would be anxious to pay off as soon as possible by hosting their own events.

The religious and political leaders must have coordinated this type of system, but the presence of high status individuals in Coles Creek society is obscure. Most Coles Creek burials in any given cemetery are much like all the others, with few individuals distinguished by associations with special artifacts or other types of burial arrangements (Kidder, 1992). I infer that the concern with differential status was more on a lineage- or kinship-based community level, and that individual leaders, especially prior to A.D. 1000, were either not interested in or not capable of using their positions to amass personal wealth and power. In short, differential status existed in these societies, but they had not become hierarchically stratified to the extent of Southeastern chiefdoms encountered by De Soto and other European explorers of the 16th century.

Chiefdoms had arisen no later than the 11th

century A.D. in the central Mississippi River valley and across the South (Barker & Pauketat, 1992; Steponaitis, 1986). Insignias of chiefly office and other high status indicators accompany burials in many early second millennium sites surrounding the Lower Mississippi Valley. Maize is usually well represented in middens, pits, and structures at these sites (Scarry, 1993), and stable carbon isotope studies on human bone usually indicate a significant amount of maize in the diet (Buikstra & Milner, 1991).

The most elaborate expression of chiefly authority is found just across the Mississippi River to the east of St. Louis, at the great site of Cahokia. More than 100 mounds were constructed at that site. The largest, called Monks Mound, stands 33 m in height. Burials in Mound 72 show the lavishness with which chiefs were interred. Grave goods include thousand of beads made of marine shell from the Gulf of Mexico or Atlantic Ocean, rolls of sheet copper from Lake Superior, mica from the Appalachian Mountains, and arrow points of various non-local cherts (Fowler, 1975). People living at Cahokia grew a great deal of maize, but crop diversity was high, and maygrass, chenopod, erect knotweed, and other native grains were more than minor supplements (Johannessen, 1988; Lopinot, 1991).

Late Coles Creek culture (A.D. 900–1200) in the Lower Mississippi Valley was affected by these developments, but persisted in established traditions for the most part. Mound centers are larger, however, indicating increased status differentiation and, possibly, consolidation of authority and responsibility by individual leaders. Our work in Tensas Parish demonstrates that maize was present between A.D. 900 and 1200, but in frequencies too low to allow an inference of large-scale agriculture. Instead, Late Coles Creek people continued to fish, hunt, and harvest wild plant foods for their primary sustenance (Fritz & Kidder, 1993).

Coles Creek culture evolved into what archaeologists call Plaquemine, with a chronological boundary at A.D. 1200. We tested a single-component Plaquemine locale named the Emerson site, which dates to approximately A.D. 1450, in order to determine whether or not agricultural intensification could be observed. We found maize fragments in abundance in the middens at Emerson: 27 times more maize per liter of soil floated than at Osceola. The relative abundance of Plaquemine maize strengthens our belief in minimal use of maize by Coles Creek people. Heavy use of acorns and wild fruits continued into late prehistoric times, but Plaquemine farmers did not incorporate native seed crops such as *Iva annua* var. *macrocarpa* or *Chenopodium berlandieri* subsp. *jonesianum* into their food production systems.

WHY DID COLES CREEK HUNTER-GATHERERS BECOME PLAQUEMINE FARMERS?

We went to Tensas Parish thinking we might find early agriculturalists growing either maize or native starchy seed crops. Instead, we found socially complex fisher-gatherer-hunters persisting in their harvesting of wild resources in spite of the fact that people around them, and presumably well known to them, were adopting or intensifying maize agriculture. Farming came late to Tensas Parish, not being intensified until at least A.D. 1200. The big question for anthropologists, still largely unanswered is: Why did hunter-gatherers switch to farming? In this case, why would they begin the arduous task of clearing levees and any other deposits of non-clayey soil, and worrying about crops sensitive to floods, droughts, insect pests, and raids by deer or other marauders?

Anthropologists of the 1990s are more interested in the role of social relations for stimulating this type of transition and less satisfied with the ecologically or demographically causal explanations that were popular during the past few decades (Barker & Pauketat, 1992; Nassaney & Cobb, 1991). Within the realm of social relations, Southeastern archaeologists are especially intrigued with high status individuals and the decisions they may have made to encourage agricultural production. Early chiefly elites in Tensas Parish would have been responsible for hosting and negotiating with their counterparts across the Late Coles Creek region and for dealing with the already more stratified Mississippian chiefs. Details of the prehistoric decision-making process will never be known, but one plausible scenario is that maize was first used primarily as a ritual offering or foodstuff at special occasions (Fritz & Kidder, 1993; Rose et al., 1991; Scarry, 1993). The more maize a community could provide at a feast, the more leverage its leaders would have in negotiating with chiefs of expansionary Mississippian polities whose civic centers had granaries full of maize.

Another plausible social mechanism, not mutually exclusive with the above, involves intermarriage between the Coles Creek hunter-gatherers and their Mississippian neighbors. Women farmers entering a village of acorn harvesters would want to feed themselves and their children the proper

way—i.e., the way they themselves had been raised—so would initiate maize cultivation. If this type of marriage initially occurred at the elite level as a political strategy, maize production would be seen as prestigious and would more likely be emulated. Because North American Indian men did little if any farming anywhere in the Eastern Woodlands, the transmission of agricultural expertise was probably from woman to woman.

Complex Hunter-Gatherers

One of the key points of this paper is that Coles Creek people, like people in the Basin of Mexico before 3500 B.C. and groups in the Tucson Basin at 2000 B.C., were sedentary hunter-gatherers on the eve of their adoption of agriculture. They maintained relatively high population densities, marked their territories with mound-dominated ceremonial centers and cemeteries, and were internally differentiated by ranked social positions. Appreciation of complexity in hunter-gatherer societies in general is growing, and most information comes from the archaeological record and from archival sources (Bar-Yosef & Belfer-Cohen, 1992; Gebauer & Price, 1992; Henry, 1985; Marquardt, 1992; Price & Brown, 1985). Some of these societies—the Natufians of the ancient Levant, for example—probably deserve credit for primary plant and animal domestication, and others were the earliest to adopt agricultural practices when exposed to them by neighbors. The transition to agriculture was not always immediate or large scale, and not necessarily an economic improvement. The sedentary hunter-gatherer lifeway persisted for millennia in several resource-rich zones even after cultigens were present in nearby regions and almost certainly available through exchange.

Classic examples of complex hunter-gatherers who flourished in North America until they were killed off or subjugated by Europeans are the Calusa in southwest Florida and the many diverse native groups of California and the coastal Pacific Northwest. All of these groups had access to abundant aquatic resources as well as to wild plant carbohydrates. The Californians harvested acorns from well-tended groves, storing and consuming great quantities of acorn meal (Jackson, 1992; Kroeber, 1976). All of these groups possessed knowledge of plant husbandry, and all probably grew tobacco for ritual purposes. Even the existence of special-purpose gardens, however, does not alter the classification of these groups as complex hunter-gatherers.

Calusa society was destroyed before 1800 by disease and social disruption brought about by Spanish missions and American colonial expansion, and most native Californians had either died, accepted mission life, or moved away from their home villages by the 1830s. In spite of rapid alteration by epidemic disease and enforced missionization, abundant evidence exists for strict social regulation in these societies (Arnold, 1992; Hann, 1991; Kehoe, 1992; King, 1978). They were not like the stereotypical Plains Indian bands in the Wild West during the late 19th century, where a person's status was achieved primarily through deeds and exploits. Chiefs along the West Coast were born into their high status positions and had great authority and responsibility. In California, status at birth determined whether one would be a boat owner, doctor, dancer, craft specialist, or mere commoner. Accumulation of wealth was a major concern, a form of shell currency was exchanged, and slave ownership was practiced until Canadian and U.S. laws made it illegal (Kehoe, 1992). Indian people of the Northwest Coast today still appreciate the ancestral social hierarchy (Blackman, 1982).

Competition existed between groups, and violent conflicts were not unusual, but a great deal of cooperative interaction took place in the form of trade and feasting. Sea transportation was carried out in boats made of redwood planks, in tule reed rafts, or dugout canoes (Ames, 1985; Arnold, 1992; Kehoe, 1992). Although group territories were restricted, the active exchange networks made it possible to distribute marine resources from offshore, shellfish from the coast, salmon from upstream spawning grounds, acorns and seeds from interior valleys, and other foods from various ecological zones. I believe that strong social regulation within and between political units was necessary for maintenance of high population densities, even in an environment as diverse as California.

Prehistoric population sizes are notoriously difficult to calculate, and published estimates diverge widely. The most reliable recent reconstruction is, in my opinion, that of Douglas Ubelaker (1988) of the Smithsonian Institution's Department of Anthropology. Ubelaker made tribe-by-tribe estimations of North American Indian populations at the time of European contact based on information compiled by regional experts for the Smithsonian's recent *Handbook of North American Indians.* California and the Pacific Northwest ranked first and second in population density, with an estimate of 75 people per 100 km^2 in California and 54 people per 100 km^2 along the Northwest Coast

TABLE 5. Estimates of North American population size and density at first European contact (from Ubelaker, 1988).

Area	Population	Population per 100 km^2
California	221,000	75
NW coast	175,330	54
Southwest	454,200	28
Southeast	204,400	22
Northeast	357,700	19
Columbia Plateau	77,950	15
Plains	189,100	6
Great Basin	37,500	4
Arctic	73,770	3
Sub-arctic	103,400	2

Total population = 1,894,350. Average population per 100 km^2 = 11.

(Table 5). The third and fourth most densely populated regions were the Southwest and Southeast, with an average of 28 and 22 people per 100 km^2, respectively. The complex hunter-gatherers of the West Coast were, according to these estimates, extremely successful at extracting the resources available to them. There is little cause for wondering why they failed to plant more crops even though they had access to them and understood the concept of cultivation.

Can We Learn from the Past?

Two years ago I met with Alwyn Gentry, who was hoping that the "ethnobotanical" part of my paleoethnobotanical research might be of some value in his conservation efforts. After telling him about such things as domestication of *Chenopodium berlandieri* subsp. *jonesianum* and intensification of native starchy seed crops along with maize in eastern North America, he smiled and said, "You're no help." His death and the consequent premature truncation of his contributions challenge me to search harder for relevance in the study of ancient plant use. The insights I offer do not rival the importance of finding cures for diseases or salvaging information from threatened rainforests. Archaeological data can, however, be used to strengthen arguments for sustainable harvesting of forest products, and can serve as alternative models to low diversity agricultural systems.

In several parts of the world, the achievements of complex hunter-gatherers of the far and not-so-far distant past highlight the feasibility and potential stability of nonagricultural systems. These societies were not mobile, egalitarian bands like the !Kung San of the Kalahari Desert (Lee & DeVore, 1968). They were sedentary and territorial, practiced food storage, engaged in widespread trade, and allowed their leaders to have special privileges and to pass these on to their children. The duration of non-agricultural complexity was not always brief, and it did not inevitably evolve into full-blown farming given the first window of opportunity. Local vegetation was undoubtedly altered by these societies, but not necessarily in an adverse or irreversible way.

The most significant aspect of a shortened history of domestication in the New World, as I interpret the recent AMS radiocarbon dates, is that it adds at least two millennia to the era preceding maize or other food crops. It turns attention away from the semiarid valleys of Tamaulipas, Puebla, and Oaxaca, where nomadic bands made seasonal rounds for thousands of years, and directs attention to more optimal zones where aquatic resources and surrounding vegetation enabled people to settle more densely. In the absence of directly dated early cultigens from rockshelter sites, the Basin of Mexico scenario acquires wider appeal.

In Mesoamerica, as elsewhere, complex hunter-gatherers may have flourished for some time (between 6000 and 3500 B.C.) and themselves engaged in primary plant domestication and subsequent intensification (between 3500 and 2000 B.C.). Farming villagers who made pottery and relied on cultigens for most of their sustenance dominated the scene by 1500 B.C. (McClung de Tapía, 1992), after a shorter transitional period than previously believed.

In Louisiana, also, the early Coles Creek mound builders and their ancestors were nonagriculturalists practicing sustainable harvesting of the rich inland Mississippi River delta. Adoption of maize came later than previously believed, and it may have been required or encouraged by elite individuals as a result of interactions with neighboring farmers. North American Indians along the West Coast and in southwest Florida did not become farmers until after European contact, and the archaeological record in these areas shows long sequences of in situ harvesting of wild plant and animal foods and early growth of social mechanisms to control sedentary life (Ames, 1985; Arnold, 1992; Marquardt, 1986, 1992).

North American Indians before 1492 did not have to cope with threats from greedy and even more complex imperialist nations or multinational corporations capable of stripping forests and orchards, polluting the waters, displacing people from their managed landscapes, and substituting a totally different ecosystem. It is impossible, of course, to

transpose ancient nonagricultural subsistence economies into today's world. Even for pure research purposes, economic valuation of prehistoric resources would require imaginative quantification. Moreover, evidence for nonsustainability can be inferred even for some ancient hunter-gatherers. Paul Martin's (1984) Pleistocene overkill hypothesis, for example, is cited by Godoy & Bawa (1993) in a recent issue of *Economic Botany* devoted to the subject of sustainable management of non-timber tropical forest products. The Pleistocene overkill hypothesis, however, is considered by anthropologists to be largely disconfirmed (Grayson, 1991). Meltzer (1993: 160) wrote, "It is now clear . . . that Paleoindian hunting was not the prime cause of, and perhaps, did not even contribute to, the terminal Pleistocene extinctions." Even if Paleoindians did play some role in the extinction of Ice Age megafauna, it does not follow that all prehistoric hunter-gatherers inevitably overexploited their environments.

Although resource depletion probably occurred in some times and places, long periods of continuity are evident. Documentable cases of persistence and stability such as the ones discussed above deserve due consideration even if conditions were not static. An appreciation of the social complexity and population densities achieved by these groups makes them seem less remote, less primitive. When seeking solutions to subsistence and conservation problems on the community level, the long archaeological record of sustainable harvesting is a source of information and inspiration.

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MEDICINAL PLANTS AS SOURCES OF NEW THERAPEUTICS[1]

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ABSTRACT

Indigenous peoples traditionally use a wide range of plants to maintain their health. Modern medicine has benefited substantially from anecdotal results of their empirical methodology by selecting needed candidates for a currently inadequate pharmacopeia to treat large numbers of illnesses. When the rapid destruction of diverse tropical forests, where the majority of cultured peoples using traditional medicine live, is related to the recent upsurge of interest in finding new antiviral, antineoplastic, and other agents, there is ample reason to justify learning what plants people use, how they use them, and under what circumstances the plants prove efficacious. These often ignored ethnobotanical findings set the stage for targeting plant materials that can be meaningfully analyzed for activity using appropriate biodirected assays and, when these are significant, for chemical isolation and characterization of active principles. Examples of ethnomedicinally selected western Amazonian plants used by Jívaro Amerindians having potential value by modern medical standards are described and evaluated.

There is great scope for new drug discoveries based on traditional medicinal plant use throughout the world (Cox et al., 1989; Farnsworth, 1984; Farnsworth & Soejarto, 1991; Moerman, 1991; Phillipson & Anderson, 1989; Schultes & Raffauf, 1990; Turner & Herbda, 1990; Tyler, 1986). In a recent review, Lewis (1992) outlined several hundred plants by medical category currently used in modern medicine and pharmacy, illustrating recent selections of natural products and their incorporation into modern pharmacopeias. He also showed how a culturally intact tribe, the Jívaro, use plants now, as they have for perhaps thousands of years, for health care on a daily basis. However, as he stated, "Serious dangers exist for the survival of such peoples and their cultures, and the ecosystems which nurture them and provide Western and traditional medicines with novel plant products for human well-being everywhere. In this race against ecosystem destruction, researchers in many disciplines must rally to provide the impetus to save global diversity while, at the same time, accelerating studies of ethnomedicine in consort with biomedical and chemical teams for developing new natural products and drugs needed by humans into the next century." As about three-quarters of the biologically active plant-derived compounds presently in use worldwide have been discovered through follow-up research to verify the authenticity of data from folk and ethnomedicinal uses (Farnsworth et al., 1985; Soejarto & Farnsworth, 1989), it is reasonable to conduct ethnobotanically directed research in order to optimize the search for novel pharmaceuticals.

This paper will examine five topics relating to ethnomedicinal targeting of plants as potential sources of new therapeutics: (1) initial collection of medicinal plants for screening, (2) evaluation of targeted collections, (3) plants as sources of drugs, (4) ethnomedicine of western Amazonian plants, and (5) intellectual property rights.

1. Collection of Medicinal Plants for Screening

There are a number of different ways to obtain plants for screening. These range from "random" or biodiversity-based selection to selections based on taxonomic, chemical, or ethnobotanical data, or any combination of these and other approaches. To date, most large plant collections are obtained "at random," with a goal of procuring as diverse a taxonomic representation as possible. The first National Cancer Institute (NCI) "random" procurement program (1960–1980) had several guidelines: a sample consisting of any plant part or combinations of plant parts would be acceptable if the sample could be supplied in an amount of 1

[1] We thank Gordon Cragg, Chief of the Natural Products Branch, National Cancer Institute, for testing crude plant extracts in the NCI's anti-HIV screen.
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pound (0.37 kg) or more, while a duplication of species would be acceptable if samples were collected in different seasons or from different geographic areas. As stated by Spjut & Perdue (1976), "The orchids, for example, are not well represented because they are usually not sufficiently common." It is obvious that in such collections small or uncommon plants are selected against. In addition, certain plant parts would also not be widely collected even though commonly used in traditional medicine, such as root bark. Not only is root bark difficult to obtain, it is also destructive to vegetation. Collecting 0.37 kg of root bark, even in consort with other plant parts, might require many hours of work. Obviously, it cannot be justified to spend too much time for one collection when a deadline is approaching to collect 500 or more samples for NCI. What would be ignored? Root bark.

Spjut & Perdue also reported that, as collecting progressed, it became evident that certain families, notably the Apocynaceae, Celastraceae, Simaroubaceae, and Thymelaeaceae, were good sources of cytotoxic activity. As a result, a special effort was made to seek out plants of these families for screening. As they said, members of the Poaceae and a few other families proved poor sources of activity, hence further collecting of these plants was discontinued. Moreover, some suppliers of plants or extracts to the NCI program concentrated on medicinal plants. Clearly, this was not intended as "random" sampling, but rather "modified random." In this first NCI program, extracts representing 20,525 species (in 4716 genera and 317 families) were screened for antitumor activity, of which 2127 species, about 10.4%, in 1225 genera (26%) were found to be active (Spjut & Perdue, 1976).

What the NCI guidelines and recommendations introduced were taxonomic, chemical, and ethnobotanical factors into "random" collecting (Spjut, 1985). The Apocynaceae, which are diverse in secondary metabolites, were collected whenever the opportunity occurred, and correctly so, for the purpose of collecting was not to obtain a random sample, but to find plants active in antineoplastic screens. Therefore, the primary screening activity of 10.4% was based on a "modified random" collection. This frequency of activity probably was much higher than would be anticipated from randomness alone, and might more closely approximate the 6% activity found by Balick (1990).

Using various literature references, Spjut & Perdue (1976) divided species tested in the NCI assays into categories, based on traditional medicinal uses. Hence, for vermifuges, 29.3% of species (52.2% of genera) were active in antineoplastic screens, a figure about three times greater than in random screening results; for fish and arrow/dart poisons, 38.6% of species (65.8% of genera) and 52.2% of species (75% of genera), respectively, were 4 to 5 times more active in anticancer screens than in random screens; and, among four selected families having known traditional medicinal uses to treat cancer (Hartwell, 1967–1971), 19.9% of species (52.4% of genera) were active, about twice as numerous as would be expected from random screen results (Table 1). The Rubiaceae, however, proved to be more than three times as active as compared to random screens (Table 2). This categorization, while indirect, nevertheless shows that specific types of medicinal plants or selected families can provide valuable leads in increasing the frequencies of activity in certain primary assays. Since primary antineoplastic screens are based on detecting selective cytotoxicity for animal cells, it follows that those plants which are known to be highly toxic, like vermifuges and poisons, will tend to be more active in such screens than most me-

TABLE 1. Primary activity frequencies of plant species used in antineoplastic screens, NCI program 1 (from Spjut & Perdue, 1976).

	% active
20,525 spp. tested, 2127 spp. active	10.4%
Subset selected by traditional medicinal use:	
Vermifuges	29.3%
Fish poisons	38.6%
Arrow/dart poisons	52.2%
Four families known to treat cancer (Table 2)	19.9%

TABLE 2. Number of species in four selected families used against cancer in traditional medicine (Hartwell, 1967–1971) screened for antitumor activity, and number and percent of active species (modified from Spjut & Perdue, 1976).

	Number/percent of species			
Family	Traditional medicine	Screened	Active	% active
Fabaceae	180	136	25	18.4
Liliaceae	89	51	10	19.6
Rubiaceae	48	18	6	33.3
Rutaceae	22	21	4	19.0

dicinal plants, which are often used because of low toxicity.

We have shown that so-called "random" collecting of large numbers of plants is not at all random. However, individual collections may be randomized, and in these instances the collector, usually a systematist, may collect material for no special reason other than it is in flower or fruit, the typical collecting bias, or that it looks unusual (i.e., unknown to the systematist). If such collections turned out active, these finds would be serendipitous. But if a plant is collected because it is known to be used medicinally, even if for a different purpose than the intended screen, then this is not complete serendipity, for it may not have been brought to the laboratory if it were not for its traditional medicinal use. The initial discovery of the antineoplastic activity of *Catharanthus roseus* (L.) G. Don f. (Apocynaceae) was made through the good fortune that reviews of the pathology of treated mice indicated an induced leukopenic state, and that it was from this observation that the value of the species in treating leukemia was derived. Yet, Noble et al. (1958) and Svoboda (1961) included *C. roseus* in their antihypoglycemic screening programs because of its well-known folk use to treat diabetes. As such, this major discovery in medicine was based, in part, on initial targeting of traditional medicine.

The discovery in the early 1970s of the antineoplastic compound taxol from *Taxus brevifolia* Nuttall (Taxaceae) in a broad or random collection program of plants from the Pacific Northwest is presumably an example of pure serendipity. It is unlikely that the collector(s) knew that an allied species, *T. baccata* L., which is now known to possess frequencies of taxol even higher than *T. brevifolia* (Elias & Korzenevsky, 1992), had long been used in Asian Indian traditional medicine to treat cancer (Hartwell, 1971, see 1967–1971). In retrospect, based on this use in southern Asia, it would not be unusual to find an American species in the same genus having a similar compound and efficacy.

One of the best recent examples representing unqualified serendipity is the discovery of active anti-HIV compounds michellamines A and B in *Ancistrocladus korupensis* D. W. Thomas & Gereau (Ancistrocladaceae) (as *A. abbreviatus*, Gustafson et al., 1992) based on the original random collection made by Duncan Thomas in Cameroon. Thomas was accompanied by local helpers who had no name for this liana and who seemed even to be unaware of its existence in the ecosystem. Although the material was sterile, Thomas correctly surmised the identity of the genus, and as he had no prior ethnomedicinal or chemical data relating to it, and only passing taxonomic knowledge or interest, the collection and subsequent finding of anti-HIV activity in *A. korupensis* is an example of unqualified serendipity. In Malaysia, however, *A. tectorius* (Lour.) Merr. is used to treat dysentery (Perry, 1980). In generic terms, severe diarrheas, like dysentery, have a variety of etiologies which include bacteria, viruses, protozoans, and intestinal parasites, thus giving credence to the hypothesis that species of the same genus with anti-infective uses are likely to possess bioactive compounds.

As a consequence of the biases of major collecting programs, there are huge gaps in our screening profiles, which include many small plants, most infrequently occurring plants, and members of families judged low in secondary metabolites based on initial results involving often fewer than 5% of species and few assays. Certainly, some exciting finds will materialize from random collecting, which otherwise may not be found, but at what cost in time, energy, and funds compared to more focused collecting?

2. Screening of Targeted Collections

In their antiviral screening program, a Belgian group (Van den Berghe et al., 1985) reported that selection of candidates for screening when based on traditional medicinal data compared to several other methods "gave a five times higher percentage of active leads," even though in some cases the same active compounds were isolated from botanically unrelated active plants. In a preliminary test using plants submitted to the NCI anti-HIV screen, Balick (1990) found that random plant collections provided 6% activity, whereas those based on ethnobotanically selected "powerful plants" by an herbal healer yielded 25% activity, a four times greater frequency. Although these results were based on small numbers and were not statistically significant ($P = 0.10$), the trend was apparent, i.e., preselected medicinal plants had a greater frequency of anti-HIV activity than randomly sampled plants. These two examples, even though quantitatively limited, indicate that ethnobotanical selection may prove four to five times superior in detecting active agents during primary screening than by following the random method.

In September 1992, 50 crude aqueous and organic extracts, prepared following the NCI extraction protocol (G. Cragg, pers. comm.), from 25 vascular plants were submitted to the NCI's primary anti-HIV screening program. Collections rep-

TABLE 3. Ethnomedicinally targeted anti-infective plants tested in the NCI primary anti-HIV screen.

	Screens	Active (number/%)	Significance
NCI modified random	16,886	1429/8.5%	
Aqueous extraction	ca. 8443	1174/13.9%	
Solvent extraction	ca. 8443	225/3.0%	
Anti-infective targeted	50	15/30%	$P = <0.001$[1]
Aqueous extraction	25	10/40%	$P = <0.01$[2]
Solvent extraction	25	5/20%	$P = <0.05$[3]
Relative antiviral targeted subset	14	10/71.4%	$P = <0.001$[4]

[1] $\chi^2 = 20.9$, df 1; [2] $\chi^2 = 8.7$, df 1; [3] $\chi^2 = 4.9$, df 1; [4] $\chi^2 = 37.6$, df 1.

resenting 23 genera in 19 families from four continents had been prescreened by traditional medicinal use for anti-infective activity. About half of these collections represented a further selection of plants based on their use in the generic treatment of a viral infection considered to be ancestrally related to HIV and in some instances validated by traditional healers and specific antiviral primary screens. These results were compared with extracts of terrestrial plants obtained randomly (= modified random as already described) and tested by NCI in the same screens used from late 1987 through October 1992 (Cardellina et al., 1993, pers. comm.) during the second modified random screening program of NCI. Of the 50 samples tested, without regard to type of ethnomedicinally reported infection or source of information, 15 samples or 30% proved weakly to strongly active against HIV in vitro. When these results were compared with the 16,886 total extracts of terrestrial plants obtained randomly of which 1429 or 8.5% proved active in NCI's primary anti-HIV screens, our anti-infective prescreened samples proved highly significantly different ($P = <0.001$) (Table 3). Considering aqueous and organic extractions separately, significant differences were also obtained between preselected and random collections: 25 aqueous-extracted samples gave 10 active extracts against HIV (40%) compared to NCI's 13.9% activity ($P = <0.01$), and 25 organic-extracted samples produced 5 active extracts (20%) compared to NCI's 3% activity ($P = <0.05$). Selective prescreening based on ethnomedicinal use is, therefore, an effective means of obtaining targeted plant materials, at least when used as anti-infectives.

Anti-infective plants used in traditional medicine to treat a virus considered to be ancestrally related to HIV were included within the above data set (Elvin-Lewis, unpublished data). When examined using the primary anti-HIV assay, 10 of 14 samples or 71.4% (7 aqueous, 3 organic) proved active. Compared to random NCI collections, this subset of specifically targeted extracts was highly significantly different ($P = <0.001$). To our knowledge this specific example, as well as those representing the whole collection, provide the first statistically significant data confirming the value of ethnomedicinal preselection as a means of primary targeting terrestrial plants for further research (Table 3).

Samples of these active screens may be further tested by NCI to eliminate polyphenols and complex polysaccharides. Regardless of the outcome of these tests, however, and whether or not unique commercially useful compounds are present, use of plants by indigenous peoples as anti-infective aqueous ingestants in whole infusions or decoctions could prove efficacious, and therefore of immense value in traditional medicine for use throughout much of the tropical world. Moreover, lengthy and costly biodirected assays followed by chemical fractionations and characterizations often lead to disappointing results when toxicities prove too great. This is not often an outcome when plant remedies utilized as anti-infectives are ingested without apparent side effects and following procedures which may have been used for generations.

We have described anti-infectives as a whole and viral subset, but another way to group these collections and their assay results is to more clearly categorize the original ethnomedicinal sources of information as either primary or secondary. Primary sources we define as information obtained from those using plant materials and/or treating with them, based on personal experience with their preparation and administration, and judgment of efficacy. By secondary sources we include information obtained from literature, herbarium collection labels, and those persons having learned of plant uses, but who lack personal experience. The usefulness of this dichotomy in showing different

TABLE 4. Activities in anti-HIV screens of plant extracts used as anti-infectives based on primary and secondary ethnomedicinal data (activity = aqueous and/or solvent extracts).

Source	Plants	Active
Primary	7	7 (100%)
Secondary	18	4 (22.2%)

$P = <0.001$ ($\chi^2 = 12.4$, df 1).

values of primary and secondary ethnomedicinal data is illustrated by the anti-HIV activity of the 25 plant collections already described. We found that all seven primary source plants were active to some degree (weakly to strongly, either aqueous or solvent extracts or both), while only 22.2% of secondary source plants were active (Table 4). The difference between them is highly significant, indicating that the best ethnomedicinal information by far is that obtained from direct contact with those persons who are using plants to improve their health. This information then translates into highly focused and targeted data of enormous potential relevance in natural products research. As Balick (1990), Cox (1990), Farnsworth (1990), Lewis (1992), Malone (1983), and Plotkin (1988) argued, ethnobotany provides the mechanism for rapid assessments of the pharmaceutical potential of species, an obviously significant procedure when there is insufficient time and funds to test the majority of species.

3. PLANTS AS SOURCES OF DRUGS

Many plants are already sources or templates of numerous new pharmaceuticals. Just how numerous is often difficult to discern, for this number should include drugs used in a chemically unmodified form, as well as template molecules used to design and generate completely new drug substances (Kinghorn, 1992). At times the chemical histories of such modifications are difficult to trace from original plants to novel synthetic compounds, but a few examples will illustrate important, if often obscured, connections.

Atropine and scopolamine, tropane alkaloids that antagonize the action of acetylcholine, are isolated from a wide range of solanaceous genera, which are particularly common in Europe and the Middle East where they have had a long history of traditional medicinal use to relieve colic, to dilate pupils of the eye, and as poisons and ingredients of witches' brews. Today these compounds and their synthetics are widely used in antispasmodic preparations, to prevent motion sickness, and also to stimulate pupil dilation. Perhaps less well known is the field of synthetic atropine and cocaine analog chemistry, in which an amino alcohol used in the manufacture of the synthetic cocaine analog dimethocaine was incorporated into a synthetic analog of atropine, leading to the production of amprotropine (Sneader, 1985). Amprotropine became the forerunner of synthetic selective hormone antagonists, including the development of antihistamines, which are now immensely popular and are hailed by those who suffer from allergies as miracle drugs. This industry is worth billions of dollars annually.

Cromolyn, a synthetic derived from khellin, a chromone extracted from seeds of *Ammi visnaga* (L.) Lam. (Apiaceae), was introduced in the 1970s primarily as a preventative for bronchial asthma and other allergic conditions (Lewis & Elvin-Lewis, 1983). The plant had been used for centuries in traditional medicine of the eastern Mediterranean for, among other uses, treating bronchial congestion (Quimby, 1953), but it was first introduced to Britain for experimental research after WWII by a medical officer who had observed its use as an inhalant among the Bedouins. Sales of this molecule have exceeded $100 million annually for two decades, and they continue to rise.

Quinine, a quinoline alkaloid originally extracted from the bark of *Cinchona officinalis* L. (Rubiaceae) and perhaps other species, had a long history of use in montane Peru to treat intermittent fever or malaria. This knowledge was given by the Aguaruna Jívaro to the Jesuits in 1630, who then widely disseminated the bark containing quinine to Europe and elsewhere (M. C. Gnerre, pers. comm.). Many synthetic substitutes have been prepared, including quinacrine (mepacrine), found superior to quinine for the treatment and suppression of malaria and its analog chloroquine with fewer side effects, as well as reducing malarial fevers more quickly. Even so, malaria is still the most prevalent tropical disease in the world, with millions of cases reported annually to the World Health Organization. In addition, some strains of malarial parasites have become increasingly resistant to the 4-aminoquinoline synthetics, particularly chloroquine, so that resistance has spread to most of the major areas of the world where malaria is endemic and has now been detected in over 60 countries. Often, but not always, resistant strains can be controlled by the natural quinine product. What is needed are novel antimalarial nuclei with new modes of action: perhaps the sesquiterpene lactone artemisinin isolated from aboveground parts of *Artemisia annua* L.

(Asteraceae) is one answer. This plant has had a long history of use in Chinese traditional medicine to treat fevers (dating from Ge Hong, 340 A.D.), and when several thousand patients in southern China infected with *Plasmodium falciparum* or *P. vivax* used artemisinin, 91% or more were clinically cured (O'Neill et al., 1985; Lewis, 1992). This folk remedy may provide the important enrichment needed today to treat malaria and its forms resistant to the quinolines.

Tubocurarine, obtained particularly from *Chondrodendron tomentosum* Ruiz & Pavón and *Curarea toxicofera* (Wedd.) Barneby & Krukoff (Menispermaceae), blocks neuromuscular transmission and thus achieves reversible muscle relaxation. Learned from many Indian tribes in South America who used a decoction prepared from the roots and sometimes mixed with other plants as dart and arrow poisons, crude extracts of curare were traded by the Achuar Jívaro in the late 1930s to provide a ready supply during the experimental phase of drug development (Lewis et al., 1988). Commercial amounts were later obtained from Indian and Mestizo sources throughout Peru and Ecuador and also from other areas during 50 years of Western medical use. The alkaloid has only rarely been used since 1982, when atracurium was introduced as a short acting muscle relaxant utilizing the (bisbenzyltetrahydro) isoquinoline structure of tubocurarine, but incorporating a hydrolyzable ester portion within the molecule (Stenlake et al., 1983). In parts of Peru wild populations of these long-lived and slow growing lianas were threatened from overharvesting during a half century of commercial exploitation with no regard for managed sustainability. Since the collapse of this cottage industry in the 1980s, frequencies of these two species should slowly increase, and thus reverse what would have been endangerment or even extinction without the introduction of synthetic atracurium.

4. Ethnomedicine of Western Amazonian Plants

Field Research and Databases

From 1982 to 1988, with students from Washington University and in collaboration with students and staff of the Museo de Historia Natural, Lima, we conducted ethnobotanical field research among four of the five Jívaro tribes (Achuar, Huambisa, Mayna, Shuar), the Candoshi, and the Mestizo populations of northern Peru and adjacent Ecuador. Ethnolinguists M. C. Gnerre and D. Fast W. were also an important component of our research for communication and for learning about the Jívaro cultures. These studies generated about 5500 collections of primary source information, largely based on a consensus of several local *curanderos* and others in each village who practice traditional medicine. Both men and women practitioners contributed data, the women often being more specialized and emphasizing pediatrics, obstetrics, and gynecology (Lewis & Elvin-Lewis, 1990; Lewis, 1992). No fewer than 125 angiosperm families involving hundreds of species have been recorded to treat a wide variety of illnesses (Lewis et al., 1987, 1988). On the other hand, *brujos* or witch doctors, who are male only and not represented in each community, and who practice largely with a few plants having hallucinogenic properties for contacting spirit worlds and for intercommunicating and telepathy, rarely are experienced using other plants for well-being.

In addition to a database covering all 5500 collections by family, genus, and species, with medicinal and other data, a second larger database has been developed for medicinal plants of South America as a whole. This compilation of about 14,500 entries of ethnomedicinal reports from the literature and unpublished herbarium specimen sources is based on our program at Washington University and the NAPRALERT database of the University of Illinois at Chicago. Included also are extensive files on phytochemical and biological activity of these South American plants obtained from current literature sources and organized with the ethnomedicinal data (Lewis, Elvin-Lewis, Farnsworth & Malone, in prep.). These anticipated volumes should be of value in selecting species used in traditional medicine for specific screens, especially when combined with our primary source data among one of the major tribal groups in the New World, which in total provides about 20,000 records of plants used medicinally in South America.

Therapeutic Potential of Specific Plants

With acculturation progressing rapidly among the Jívaro, much of the knowledge they possess may be lost before we are able to test even a fraction of what they use medically. This is important not only because such a loss would affect their long-term traditional health care, and thus make them even more dependent on Western medicine in the future, but it would prevent the introduction of potentially valuable medicinals into both Western medicine and traditional medical systems elsewhere. We shall examine a few examples from their pharmacopeia.

1. Wound healing Croton *(Euphorbiaceae)*. Early in our field research we observed the Jívaro taking sap from slashed stem trunks of *Croton lechleri* Muell.-Arg. (*uruchnumi*, cotton tree) and using it topically to treat cuts, abrasions, and other wounds or bites. We later learned that the sap, called *sangre de drago* (*grado*) in Spanish, obtained from this and other species of *Croton* was widely used in South America to heal various types of wounds. It was readily available from vendors on the streets of Lima and in markets throughout the region, to such an extent that this sap is undoubtedly the most commonly used traditional medicine in Peru. The tree is relatively common in both secondary and primary forests in the low eastern Andes and much of the upper Amazon basin, and as it need not be felled to obtain sap, overexploitation is not a concern in areas of Peru familiar to us.

We brought sap from Peru for testing, and our results have recently been published (Porras-Reyes et al., 1993). In brief, we found that the sap contains an active wound-healing aporphine alkaloid known as taspine. Healing properties were tested using different topical concentrations of taspine by the paired rat surgical incision model. Samples harvested at 5, 7, and 12 days post-wounding were examined for maximum breaking strength (MBS) and histology. Those treated with 250 μg of taspine, but not those with 50 μg or 10 μg of taspine, had significantly higher values for MBS than paired controls (26% by day 5 ($P = <0.005$) and 30% by day 7 ($P = <0.001$)). There was no difference between controls and taspine-treated rats at day 12. These values showed that healing occurred up to 30% more rapidly when using 250 μg of taspine compared to controls, low taspine concentrations, and 12 days post-wounding. Histological samples treated with 250 μg of taspine showed higher cellular infiltration and granulation, including fibroblast proliferation, at days 5 and 7. There was no difference between controls, those at day 12 post-wounding, or those treated with only 50 μg and 10 μg taspine.

We soon realized that the whole sap was an essential component of this useful alkaloid, for it provided the delivery system for topical application. Without it, the high insolubility of pure taspine presented a serious problem. Recently, we produced a semisynthetic of taspine with broad solubility and similar if not improved healing of wounds using our rat model. Chemical and biological studies are continuing.

2. Oxytocic Balansia *(Clavicipitaceae) parasitizing* Cyperus *(Cyperaceae)*. Jívaro women use *Cyperus articulatus* L. var. *nodosus* (H. & B. ex Willd.) Kuek. or *C. prolixus* HBK (*piripri*) tops to make crude infusions, which are drunk as oxytocic agents to aid in parturition or postpartum contractions and to reduce bleeding after childbirth. Sedge tops are essentially inert, but close examination of what the women use revealed a parasitic fungal sclerotia in apical sedge infructescences, which proved to be *Balansia cyperi* Edgerton (Lewis & Elvin-Lewis, 1990). This fungus is classified in the Clavicipitaceae, a family containing only one other genus, the well-known *Claviceps* or ergot of temperate regions. Jívaro women have, therefore, selected the only close relative of ergot found in their tropical ecosystem and used it for the same purposes as midwives did in European folk medicine centuries ago and as we do today in modern medicine. "Circumstantial evidence strongly suggests the presence of biodynamic principles similar to the ergot alkaloids in *B. cyperi*. Should this prove true, then the selection by Jívaro women of a plant parasite to aid in obstetrics is another sophisticated example of human ingenuity using empirical methodology" (Lewis & Elvin-Lewis, 1990). Within a year of this publication, Plowman et al. (1991) proved the existence of alkaloids in *B. cyperi* sclerotia similar to those of ergot alkaloids. There are numerous other examples, for these people, using plant medicines based on empirical selections, have found effective means of treating many of their illnesses.

3. Antimalarial plants. Root infusions of a number of species are drunk daily by Achuar Jívaro to treat malaria. To test efficacy, crude extracts were sent to the University of London School of Pharmacy to be evaluated for their ability to inhibit the incorporation of tritiated hypoxanthine into multidrug-resistant *Plasmodium falciparum* (K1 strain) (O'Neill et al., 1985; Phillipson & O'Neill, 1986). IC_{50} values ranging between 15 and 30 μg/ml^{-1} were not highly active, but nevertheless worth pursuing, and further purification could lead to fractions with higher IC_{50} values (J. D. Phillipson, pers. comm.). Three of five species used as antimalarials by the Achuar showed weak to moderate anti-*Plasmodium* activity. A positive frequency of 60% with even modest activity against a *Plasmodium* multidrug-resistant strain is a striking example of Indian use of plants to treat widespread malaria in the upper Amazon basin, which may provide some protection against malaria for a population without recourse to quinine bark or antimalarial compounds.

4. Stimulating Ilex *(Aquifoliaceae)*. The last example of ethnomedicinal selection by the Jívaro

relates to the ritualistic use of *Ilex guayusa* (Lewis et al., 1991). Early every morning Achuar men drink up to 2 liters of a hot leaf decoction of this holly, and after about three-quarters of an hour each man vomits profusely. Vomiting is not due to emetic properties of the decoction, as might be suspected, but to a learned ritual associated with reducing the physiological effects of the liquid and also in providing recognition of passage from boyhood to manhood. We found the trimethylxanthine caffeine in all leaf samples, with concentrations ranging from 1.73 to 7.57% dry weight/weight, the maximum value representing the highest concentration of caffeine reported for any plant material. This tree and the one with 3.95% caffeine were avoided by the Achuar as "bad *wayus*," for even after emesis, when about half of the caffeine drunk is eliminated, these concentrations were still sufficiently great to leave the consumer high from too much caffeine. The person was nervous, irritable, and might even hallucinate, but after drinking *wayus* with lower caffeine concentrations, followed by emesis, the desired stimulating effects of caffeine use were obtained and a good day had begun. Boys, girls, and women were allowed to drink what was not used by the men, and boys became participants in the morning ritual once they had entered puberty. At some time in the past, the Jívaro were able to find this major stimulant in their ecosystem and to adapt its use culturally and physiologically in unique ways.

5. Intellectual Property Rights

As the majority of our plant collections include primary ethnomedicinal data targeted by contributors for specific medicinal uses, good faith requires that such information be recognized as intellectual property in a substantive way. We believe that access to such indigenous resources and the protection of intellectual property are complementary concerns. Therefore, an obligation exists to protect the interests of contributors, their villages or tribes if the knowledge is generalized, and the country of origin, so that should a product be produced and marketed or be used as an investigative tool in drug development or testing, then royalties, licensing fees, and other forms of compensation must be provided to these individuals, their communities or tribes as appropriate, and to the host country. If communication with local contributors or their tribes is difficult, e.g., the Achuar of Peru who have limited means of handling funds and use a language that is currently unwritten with very few non-Achuar understanding it, a trust fund must be established to be used for specific purposes to benefit individuals and the tribe. The host country will also be compensated through support of their scientific and cultural institutions. As Vice President Al Gore (1992) recently wrote, participating countries need investment of funds, while investing countries need to acquire genetic resources. These can be compatible endeavors.

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TOWARD AN INTEGRATION OF EVOLUTIONARY ECOLOGY AND ECONOMIC BOTANY: PERSONAL PERSPECTIVES ON PLANT/PEOPLE INTERACTIONS[1]

Jan Salick[2]

Abstract

Plant/animal interaction is a well developed field of investigation in evolutionary ecology. Plant/people interactions have been less fully explored, yet warrant parallel investigation, incorporating ecological genetics, population ecology, chemical ecology, and community and landscape ecology. To illustrate the ways in which people and plants interact and how these affect their coevolution, I use my research on: wild and domesticated cassava (*Manihot* spp., Euphorbiaceae); wild and semidomesticated cocona (*Solanum sessiliflorum*, Solanaceae); Amuesha farming and forestry systems; campesino and natural forest management of non-timber forest products in the Rio San Juan basin, including population studies of ipecac (*Cephaelis ipecacuanha*, Rubiaceae); and conservation, from germplasm conservation to landscape planning. Interrelating socioeconomic and biophysical research—the reciprocal people/plant perspectives—needs further development.

Plant/animal interactions have been studied extensively within the framework of evolutionary ecology (Ehrlich & Raven, 1964, 1967). An elaborate body of theory, methodology, analysis, data, and interpretation has been developed incorporating ecological genetics (Ford, 1964), population ecology (Begon & Mortimer, 1981), chemical ecology (Rosenthal & Janzen, 1979), community and ecosystems ecology (Whittaker, 1975), and more recently landscape ecology and global change.

Still, evolutionary ecological investigation of plant and animal interactions needs to better integrate people. Irrefutably, people affect plant populations and plant populations intensely affect people. This separation of people from animals in evolutionary theory dates back at least to Darwin. Although, using domesticated animals extensively to document evolution (Darwin, 1868), Darwin habitually differentiated between natural selection and artificial selection.

In evolutionary theory (Strickberger, 1968), the variety of mechanisms responsible for modifying the reproductive success of a genotype is known as selection. Human knowledge of certain aspects of selection is prehistoric, including people's attempts to modify the plants and animals upon which they are dependent. Since the reproductive success of these domesticated organisms is determined by people's conscious selection of the parents of each generation, this mechanism of selection was named artificial selection. By contrast, organisms whose reproductive success is not determined by human choice are considered to be subject to natural selection.

These are artificial distinctions within the same selection process, resting on a past conviction that human consciousness is something apart from nature. Does an orangutan rejecting a less tasty durian do so any less consciously than a person selecting a tasty one to plant? Consciousness aside, is an ant defending an acacia qualitatively different from a person laying fence or shooing crows? Are not people (from a plant's perspective) really herbivores and dispersal or defense agents, exerting

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FIGURE 1. Cocona (*Solanum sessiliflorum* Dunal) is an underexploited tropical crop with (a, left) tomatolike fruits and (b, right) eggplantlike foliage.

selection pressures regardless of consciousnesses? Additionally, people's selection is not always conscious. We have not intended to select for resistant malaria. We have not intended to select for plant breeding systems (see below). But we have. Human selection is not artificial or unnatural.

From this perspective, economic botany is primed for a "new synthesis" (Fisher, 1930), for an integration of ecological and evolutionary economic botany. I have been exploring simple possibilities for the last ten years: basically, reinterpreting plant domestication, cultivation, and management as plant/people interactions.

Plant Domestication: People Affecting Plant Genetics

Plant domestication has been defined as the genetic alteration of plants brought about by the activities of people (Simpson & Conner-Ogorzaly, 1986). Such genetic alteration can be studied using the prescribed methods, analysis, interpretation, and theory of the new synthesis. These methods and analyses were the basis of a study of crop domestication and evolutionary ecology of Cocona (*Solanum sessiliflorum* Dunal) (Salick, 1992c; see also Salick, 1990, 1992b; Salick & Merrick, 1990).

Cocona (Fig. 1) is an underexploited tropical crop (Vietmeyer, 1986). Rustically domesticated varieties co-occur with wild phenotypes. These cross and produce fertile offspring. People actively select among offspring for spineless varieties (consciously) and (less consciously) for fruit qualities: since indigenous people seldom plant cocona, but rather let effective human dispersal by ingestion do its task, fruit are selected by being eaten. In contrast, spiny cocona growing under cultivation are actively weeded out of future gene pools. In the wild, cocona are small fruited and spiny. Domestication is an ongoing process, appropriate for population genetic investigation.

Additionally, the genetics are amenable. Spines were found to be one-gene dominants, which readily function as field markers. In experimental gardens, gene flow was measured. In reciprocal transplant field experiments selection by people, other herbivores, and composite mortality factors were measured in gardens, pastures, and riverbanks, respectively. These variables are the basis for a Hardy-Weinberg model (Falconer, 1981) of co-

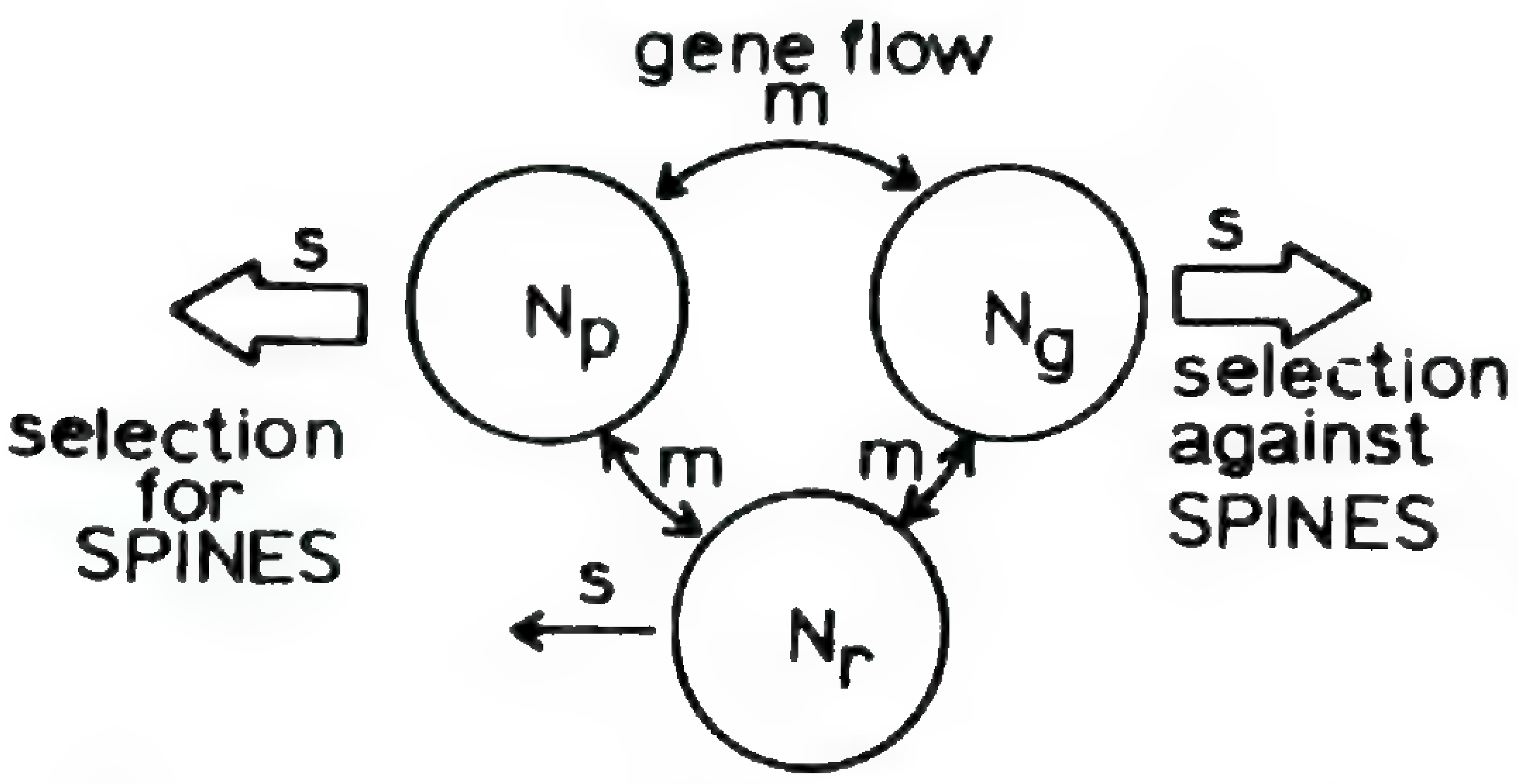

FIGURE 2. A simple Hardy-Weinberg model for cocona domestication based on population genetics of spines. There are three populations of cocona (N): in pastures (p), swidden gardens (g), and along riverbanks (r). A single gene for spines flows (m) among these populations, while selection (s) acts against spines in gardens and along riverbanks, and for spines in pastures. Selection is heavy in pastures and gardens, represented by large arrows dominating gene flow and allowing equilibrium well skewed toward the selected trait.

cona domestication (Fig. 2), the predictions of which were not significantly different from the results measured in field populations.

Nonetheless, upon testing the assumptions of the Hardy-Weinberg model, it was obvious that, as in so many *Solanum* species, cocona is highly inbred. The result in the model is to demonstrate tremendous opportunity for genetic drift and allele fixation in field populations. Not only is this a complicating theoretical result, it is also somewhat difficult for the cocona conservationist. One cannot expect to sample populations of cocona to represent its biodiversity since nearly each plant is a population in itself.

The population genetics of cocona fruits and their domestication were strikingly different from those for spines in the same organism. Cocona fruit characters are mostly maternally inherited (Fig. 3). Fruits from controlled diallel crosses were like those of fruit from the maternal plant regardless of pollen from which they were fertilized.

In general, single parent inheritance, be it variously by sexual or cytoplasmic inheritance or by vegetative reproduction, is the rule in cultivated plants (Table 1). People must select (unconsciously) for plant breeding systems. Human selection would favor breeding systems that facilitate selection, domestication, and cultivation. People may select unintentionally but effectively for simple inheritance patterns. Cocona demonstrates simple inheritance by a single dominant gene (spines), inbreeding (the entire genome), and maternal genetic inheritance (fruit size and shape). Other crops have simple inheritance by apomixis, selfing, and vegetative

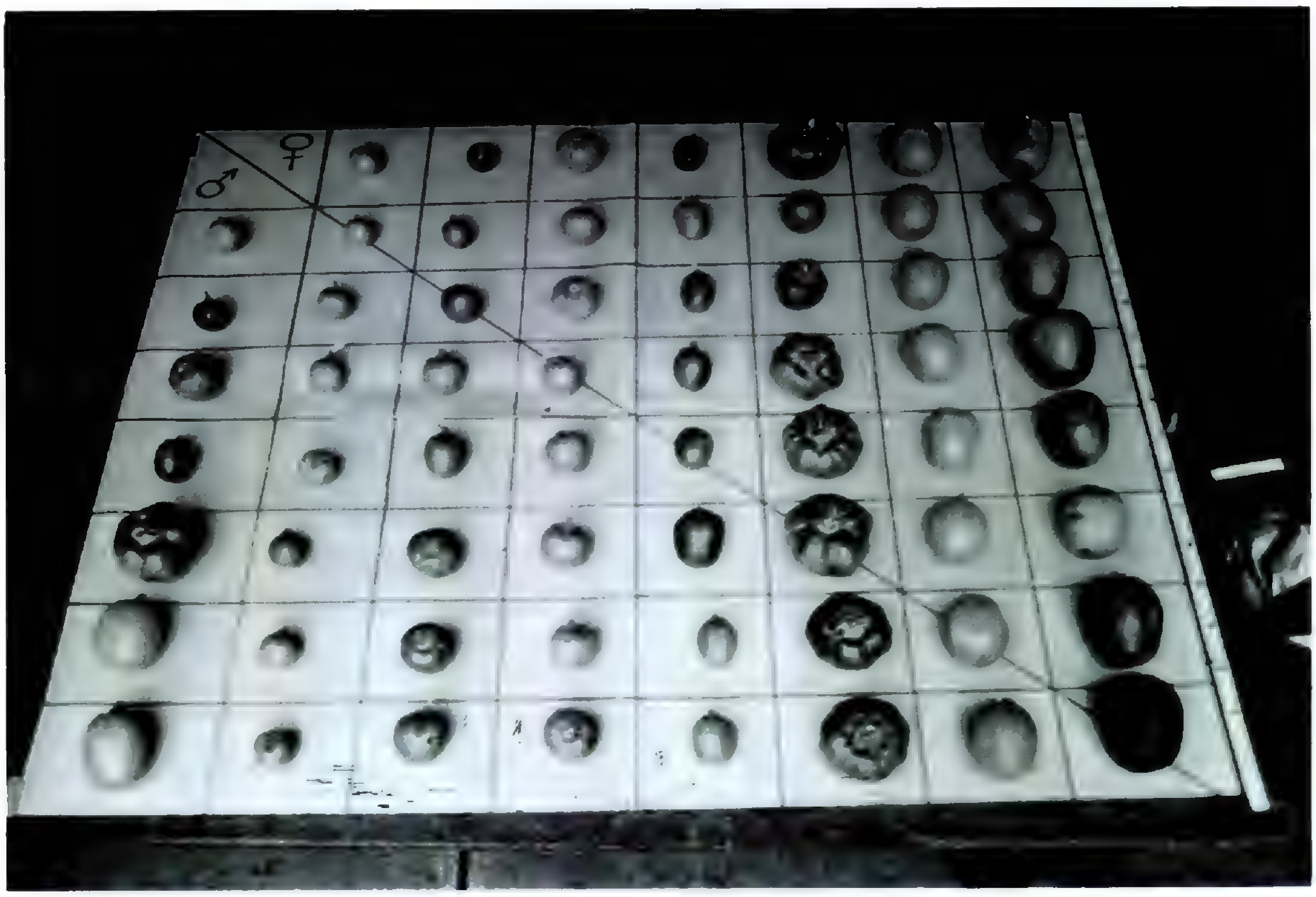

FIGURE 3. Cocona fruit characters are mostly maternally inherited as demonstrated by a complete diallel cross among seven varieties of cocona. All varieties were crossed with all varieties, including self-crosses marked by the diagonal line. Female parents are in the top row and male parents are in the left column. Progeny of any given female and male are found in the box corresponding to the column and row of its female and male parent, respectively. Parents and progeny are illustrated with representative fruit types. The significant result of the diallel is the overwhelming predominance of maternal inheritance for fruit characters.

TABLE 1. Types of uniparental inheritance in crops (both sexual and asexual). Uniparental inheritance has two main advantages: selection of desired characteristics is easy, and multiplication of the desired varieties is rapid.

True seed propagation	Vegetative propagation	Biotechnology
Selfing	Tubers	Meristem culture
Apomixis	Roots	Embryo culture
Autogamy	Cuttings	Rapid multiplication
Cytoplasmic inheritance	Stakes	Cloning
Mitochondrial DNA	Grafting	
Chloroplast DNA		

reproduction or, alternatively, may be reproduced through biotechnology (and have simple inheritance) by rapid multiplication. As our appreciation develops for the range and complexity of traits affected by natural selection, our vision of the effects of human selection and crop domestication likewise will expand.

PLANT CULTIVATION: PEOPLE AFFECTING PLANT POPULATION BIOLOGY

Plant cultivation has been defined as people "caring" for plants (Simpson & Conner-Ogorzaly, 1986). Caring ranges from minimal to intensive, from merely encouraging essentially wild individuals to careful planting and rearing of selected species. Cultivation and management can be differentiated, the former is related to plant species or populations, while the latter is plant community, system, or landscape oriented.

Cultivation can drastically affect plant population biology. Experiments documented changes in life histories, growth, production, and reproduction brought about by cultivation (Salick, in prep.). A plant species that grows in the wild and that is collected from the wild to cultivate was used in

FIGURE 4. Ipecac (*Cephaelis ipecacuanha*, Rubiaceae) is an understory tropical herb of economic importance because of its rhizomous production of alkaloids, which cause vomiting and can be used to avert poisoning. It is (a, left) collected from the wild or (b, right) rustically cultivated in forest or agroforestry beds.

these experiments, so that human selection toward domestication would be minimized. Additionally, given the previous results on the importance of direct inheritance, a plant species was selected that reproduces both sexually and vegetatively.

Ipecac (Fig. 4; *Cephaelis ipecacuanha* (Brot.) A. Rich., Rubiaceae) is an understory tropical herb of economic importance. Its roots are harvested for alkaloids that cause vomiting. The predominant alkaloid, emetine, is used to avert poisoning. It is collected from the wild or rustically cultivated in forest or agroforestry beds. Both in the wild and in cultivation it reproduces from seed and vegetatively. The experimental questions are, "How have people manipulated the population biology of this plant and why is it cultivated rather than merely collected or managed in the wild?"

Replicated, even-aged ipecac populations were grown in the tropical rainforest and under cultivation (Fig. 4) both from seed and from vegetative material. Vegetative reproduction from roots, stems, and growing tips was compared, although here data from seed and vegetatively sprouting roots are considered. The report on the two year data set on ipecac is still in press, but some clear trends can be summarized.

Plant height is greatest with vegetative cultivation (20 ± 5 cm), compared to sexual cultivation (11 ± 3 cm) and to vegetative reproduction in the wild (5 ± 2 cm). (Mortality was so high in sexually reproducing populations in the wild that no mature plant data are available.) Mean leaf area per plant differed greatly: 710 cm^2 with vegetative cultivation, compared to 110 cm^2 with sexual cultivation, and to 6 cm^2 for vegetative reproduction in the wild. Total plant biomass and root yield was greatest for vegetatively reproduced plants under cultivation (53.2 ± 15.2 g and 26.7 ± 6 g, respectively) compared to sexually reproduced plants under cultivation (25.7 ± 6.1 g and 18.6 ± 4.7 g), and vegetatively reproduced plants in the wild (3.5 ± 1.5 g and 2.5 ± 0.8 g). Flowering plants were only found among those vegetatively cultivated (75% ± 8). Death rate was highest among sexually reproducing plants in the wild (86% in the first year and 100% in the second year), compared to sexually reproducing plants under cultivation (66% year 1, 30% year 2), and vegetatively reproducing plants in the wild (43% year 1, 34% year 2) and vegetatively reproducing plants under cultivation (17% year 1, 25% year 2).

Thus, cultivation by vegetative reproduction increases ipecac plant growth, production, and reproduction and reduces mortality. Population ecology provides methodologies to address economic botany inquiry into these basic processes.

Plant Management: People Affecting Plant Communities and Landscapes

My work with plant management techniques and results deals with both agricultural and forestry management, their interactions, and to a lesser degree a larger landscape management. I have concentrated on both indigenous and campesino management techniques and more formal, technical methods of plant husbandry (and wifery—see gender in Amuesha agriculture, below).

Much attention has been given to intercropping and diverse host plant communities as a defense against insect herbivores (Risch et al., 1983). As evolutionary ecology might predict, some insect populations are adapted to such spatial diversity, however, and respond to other variables, for example, temporal diversity. Such turned out to be the case with cassava and the cassava lacebug.

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is the traditional lowland Amazonian staple starch crop. It is grown throughout much of tropical Latin America and Africa as well as Asia below an elevation of approximately 2000 m. Traditional agroecological management of cassava often involves intercropping (Salick, 1983a, 1989a, b; Salick & Merrick, 1990). Wild cassava (*Manihot* spp.) also grows in diverse communities (Salick, 1983b; Salick & Merrick, 1990). Cassava lacebug is an herbivore adapted to this spatial diversity. My studies confirm that the herbivore is more abundant on cassava growing in diverse communities.

In contrast, temporal diversity achieved through crop rotation thwarts the poorly dispersing cassava lacebug. Wild cassava is perennial, while domesticated cassava is usually grown as an annual. Cassava lacebug is a pest on the perennial but is not adapted to annual relocation. When cultivated cassava is grown continuously, planted year after year in the same field and thus simulating a perennial host, cassava lacebug thrives; while annual cassava managed in rotation with other crops (temporal diversity) defeats the herbivore. Such rotations characterize indigenous management of the annual cassava (see below, Salick 1989a, b; Salick & Merrick, 1990).

Cassava also provides a clear example of a reciprocal human adaptation to a chemical plant defense system. Cassava with variable cyanide and tannin contents is amenable to chemical ecological analysis (Salick, 1983a). The human interactions within this chemical ecology are intriguing since people have not only selected cassava with low defensive chemical contents ("sweet" varieties), but also have maintained the defensive chemicals

FIGURE 5. Plant community management is characteristic of indigenous agriculture. Agroecological management by the Amuesha of the Peruvian upper Amazon includes systems adapted and timed to extremely high rainfall patterns, and differing with soils.

in "bitter" varieties, evolving human behavioral mechanisms to extract these chemicals before use (processing bitter cassava is well described by Roosevelt, 1980). To develop studies on plant/people interactions more such reciprocal cases must be documented.

Plant community management is characteristic of indigenous agriculture (Fig. 5). The Amuesha (or Yanesha) of the Peruvian upper Amazon (Salick, 1989a, b; Salick & Lundberg, 1990; Salick & Merrick, 1990) have agroecological systems adapted and timed to extremely high rainfall patterns, and differing with soils of seasonally flooded beaches, alluvial floodplains, and acid terraces and hills (Fig. 6). Additional systems are found on continually cropped lands and in permanent home gardens. Intercropping and staggered rotations are ubiquitous within cropping systems. High, alluvial floodplains, for example, are planted with maize followed by cassava in transition to plantains. Perennials are relayed into annual crops forming agroforestry systems: fruit trees are planted under plantains and left uncut in the ensuing utilized and lightly managed fallow. All of these stages include a great variety of interplanted, minor crops. An extended Amuesha family cultivates simultaneously various fields at several stages using different cropping and even forest management systems (see below). In this way, plant community management takes on a larger landscape aspect, particularly when extended families interact and plan within an indigenous community.

Plant community management is characteristic also of natural forest management (Matthews, 1989), forest management for nontimber forest products, and indigenous and campesino forest management (Salick, 1992 a, d, f; Salick & Offen, 1992). As indigenous agriculture can be contrasted with modern monoculture (Salick & Merrick, 1990), so too can these traditional forms of forest management be contrasted with plantation forestry or with monospecific market development of nontimber forest products (Clay, 1992). Local peoples conserve and manage a diversity of forests for a diversity of products (Salick, 1992a, d, f; Salick & Offen, 1992), using a diversity of management techniques including indicator species, inplanting, transplanting, weeding, cultivating the soil, mulching, selective cutting, and liberation of desired species (Salick 1992d, f). Diversity is the mark of indigenous and traditional plant community management.

Again, plant community management can take on a larger landscape aspect with natural forest management. This may occur when indigenous or campesino families interact within a community

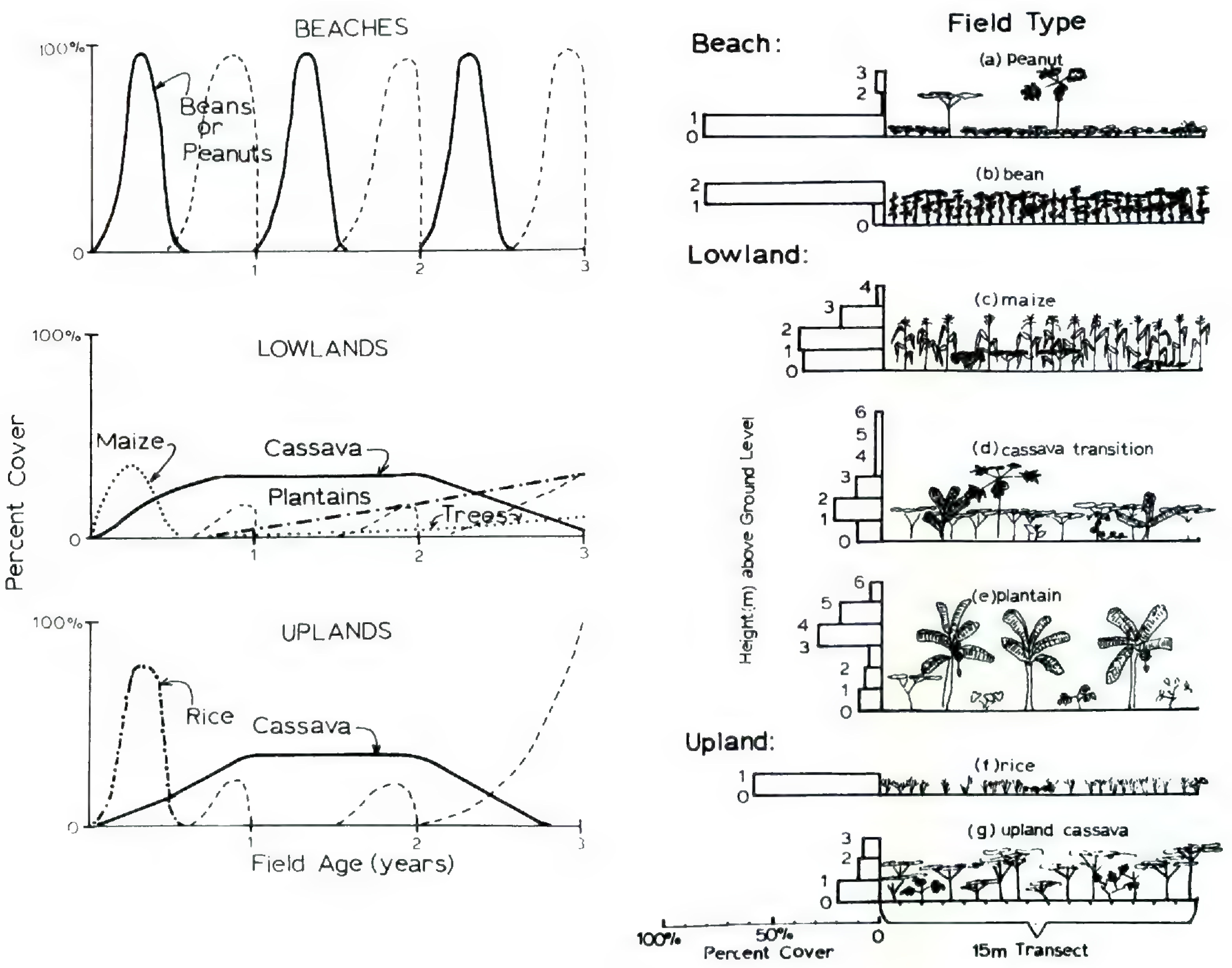

FIGURE 6. Topics in community ecology are very relevant to the sampling and analysis of traditional agriculture. (a, left) A three year time series analysis of Amuesha agricultural fields on three land types: seasonally flooded beaches, alluvial floodplains (lowlands), and acid terraces and hills (uplands). Fallows are marked with dashes (- - -). Only dominant crops are labeled although minor crops are prevalently intercropped, especially in the lowlands. (b, right) Transects (15 m) of the six field types—three land types with sequential rotations as in (a)—with average percent cover at each height class.

planning framework, or when silvicultural forest management becomes a part of conservation or regional planning (Fig. 7; Salick, 1992d, f).

THE HUMAN SIDE OF PLANT/PEOPLE INTERACTIONS

Some plant/animal interactionists bound their field to include only cases where both plants act on animals and animals on plants (coevolution sensu Janzen, 1980). So far, cassava processing aside, I have concentrated on the effects that people have on plants rather than the reciprocal. People/plant perspectives diverge from evolutionary ecology; they need to be developed through collaboration with social scientists. Two examples illustrate this difference.

Although it was not difficult to analyze human ecological patterns in Amuesha indigenous agriculture (above and Salick, 1989a, b), interpreting the immense variation and change in these patterns drew together interacting ecological and sociological variables (Salick & Lundberg, 1990). The reasons for the impressive variability in Amuesha agriculture included a range of sociological factors from complex family histories to simple differences of opinion. In particular, gender and marital status were outstanding in the analysis of Amuesha agriculture (Salick, 1985, 1992e). Often when there was an exception to a trend, or an outlying data point, it represented a single woman. Among the Amuesha, single women practice agriculture differently and opportunistically construct subsistence strategies differently than most tribal members.

Traditional systems for the extraction, marketing, and tenure of nontimber forest products based on exploitation do not favor sustainable management of tropical forests, and less, the people directly responsible for that management—the ex-

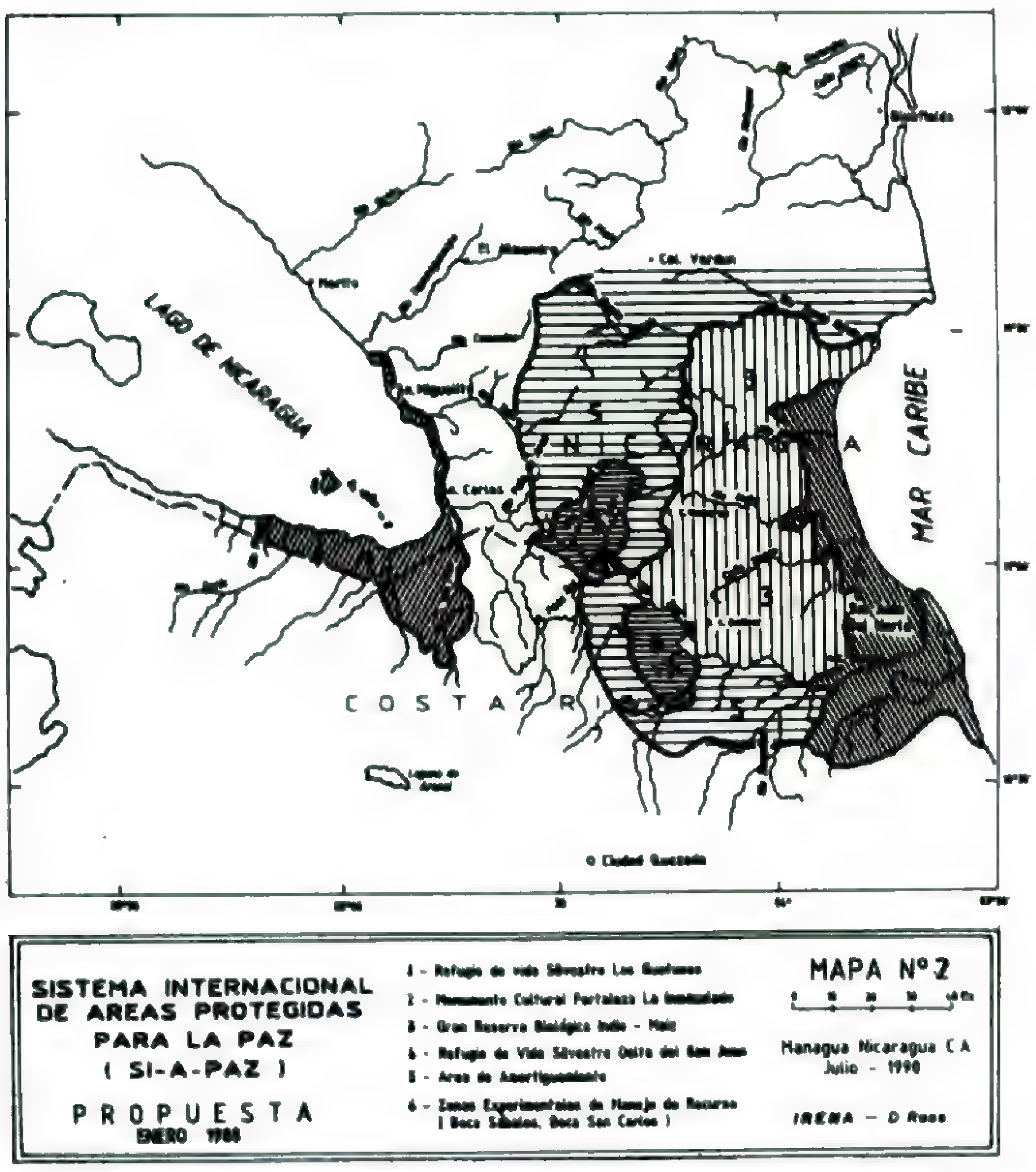

FIGURE 7. Plant community management can take on a larger landscape aspect, including natural forest management as a part of conservation or regional planning. In the International Peace Park (Si-a-Paz), Nicaragua and Costa Rica, the detailed park proposal includes (5) a buffer zone and (6) an experimental zone where our natural forest management experiments are located. (Map produced by IRENA.)

tractors. Further analysis (Salick, 1992d, f; Salick & Offen, 1992) uncovered additional confounding sociohistorical considerations, all well outside the purview of evolutionary ecology.

These attempts to develop economic botany from the human perspective are examples of the limits of evolutionary ecology in analyzing how plants affect people. Interdisciplinary collaboration seems the most productive line of investigation. Yet, the applications of evolutionary ecology to plant/people interactions have potential in determining how people affect plants.

CONCLUSION

Economic and ethnobotany interpreted as plant/people interactions can take advantage of many developments in evolutionary ecology. Within ecological genetics, people act toward plants as selective agents. Within population biology, plant life histories and their patterns of mortality, reproduction, and survival are greatly altered by people. Within chemical ecology, people both change and take advantage of plant chemical defenses for their benefit. Much of traditional plant management, both in agriculture and forestry, takes place at a plant community and landscape level. People manipulate many plant community variables that greatly affect plant evolutionary ecology. All of these areas of study have well developed methodologies, analyses, and theory, which can greatly enhance a synthetic economic botany. Undoubtedly, other subdisciplines within evolutionary ecology can yield equally productive integration.

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ECONOMIC BOTANY, CONSERVATION, AND DEVELOPMENT: WHAT'S THE CONNECTION?[1]

Janis B. Alcorn[2]

ABSTRACT

The frameworks commonly used for understanding the causes of biodiversity depletion are inadequate. This paper offers some alternative frameworks for understanding biodiversity depletion, conservation, and the roles of states, local communities, and economic botanists. Wild resources are economically important to many rural communities, and a wide range of common property tenurial systems for managing and allocating those resources have been developed. Economic botanists have documented wild plant resources, their uses, and their management. This information has primarily been used by people outside local communities who are interested in commercial enterprises and economic development. But by not paying attention to the question of tenurial rights over natural areas, wild resources, and knowledge about those resources, economic botanists indirectly participated in colonial expansion and the marginalization of indigenous peoples by neocolonial states. Today, economic botanists have opportunities to participate in efforts to recognize and document property rights to land and forest. They also have opportunities to recognize and support intellectual property rights over medicinal plant knowledge and crop varieties, although the potential application and conservation benefits of intellectual property rights are as yet unclear. Efforts to link conservation of biodiversity with development are stimulating collaborative partnerships among economic botanists, social scientists, ecologists, governments, and local communities.

The linkages between economic botany and economic development are generally conceived as linkages through knowledge about crops: major crops and their domestication and evolution; and new crops that could be promoted for the development of new products. Discovery of wild plant products that could be commercially exploited has been a standard second dimension of that linkage. The linkages between economic botany and conservation are less often considered, perhaps because the production of economically valuable plant products, particularly crops, is tied to habitat conversion which generally reduces biodiversity levels. Linkages between economic botany and conservation have primarily been forged by researchers interested in the conservation of crop genetic resources. A negative conservation linkage commonly recognized is the pattern of depletion of plant populations because they are economically valuable.

Edgar Anderson, who is honored at this symposium, showed us that it could be difficult to see what you are looking at, that there could be unexpected order in what seems disordered or unimportant, that it is easy to overlook the obvious. In this paper, I focus on something that has generally been overlooked by botanists working in the tropics, and is hence an unseen dimension of the linkages between conservation, development, and economic botany. Specifically, I focus on how rights to "common property" resources have been invisible to economic botanists and how that vision is changing. Recognition of common property management systems in turn leads us to recognize the positive linkage between conservation and economic botany—traditionally communities have been concerned about conserving plants that are valued locally for economic and other benefits.

I hope that my paper, like much of Edgar Anderson's work, is a bit controversial and that it stimulates the reader to think about the issues I raise. Most of the world's biodiversity lies outside protected areas and is under increasing threat. I

[1] I thank the organizers of the symposium for the invitation to speak. This paper summarizes a portion of a larger work in progress, a book entitled *Ethnobiology: Community, Culture, and Conservation* to be published by Columbia University Press. I thank all those who have commented on parts of that longer manuscript. In particular, I thank Owen J. Lynch for teaching me to pay closer attention to tenurial rights. I assume full responsibility for this paper. The conclusions expressed herein should not be attributed to World Wildlife Fund or to the U.S. Agency for International Development. I dedicate this paper to the memory of my father, Bruce F. Bristol, who passed away on August 15, 1993.

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make generalizations based on my own and others' research, and I draw on discussions with people in the field. Making generalizations can be risky business. Generalizations blur the rich detail of case-specific situations and mask variation. They simplify complex situations. Exceptions can always be found. Nonetheless, generalizations can be valuable, because they also offer a new model against which to compare specific cases. Such comparisons can result in revised and refined generalizations. Whether we realize it or not, we are already framing our understanding of new situations based on existing generalizations that have not been made explicit. In this paper, I try to identify the existing frameworks and make an effort to define a more useful one. Given the urgency of the biodiversity crisis, it behooves us to step back and consider whether general patterns offer lessons that might improve conservation efforts.

Tropical Forests: Their Use and Management

The tropical forests that we botanists love are beautiful and varied. The diversity of the dry forests of Africa, the mangroves of Central America and Southeast Asia, and the rainforests of Amazonia inspire us to understand them and their component species. But sometimes we forget that people live in these forests. They have lived there for thousands of years, and they live there now. These forests are their homes and the source of their livelihoods. While global maps of tropical forests show vast expanses of green belts around the Earth's equator, field biologists know you do not find unbroken primeval forests throughout this belt. Tropical forests vary greatly in size. They may be large or small, blocks or narrow, fingerlike corridors along rivers or ridges. At one end of the size scale stand the forests of Papua New Guinea, Indonesia, Central Africa, and Amazonia, where large swathes of forest occupied by indigenous peoples are being opened by loggers, oil companies, mining operations, and resettlement programs. In some places, these forests are highly diverse biotic communities; and in other places, the forest is very disturbed but nonetheless undergoing a natural process of succession. At the other end of the scale are the smaller forests of Ghana, Thailand, and Mexico, for example, which range from a few hectares to hundreds or thousands of hectares that stand as islands in land under nonforest use.

An estimated 200 to 500 million people live in the midst of the world's tropical forests (Lynch, 1990), and they use the forests and forest plants. Most of these forest-dwelling people are also farmers. They grow cereals like rice and maize, or root crops, like manioc, sweet potato, taro, and yams. Regardless of their main crop, they all depend on forests for material goods, food, and the definition of their identity. Indigenous forest people's dependence on forest and field is evident in ritual exchanges of field and forest products. For example, when the Bora of the Peruvian and Colombian Amazon have a ceremony or meeting that includes two different communities, the two ritually exchange manioc bread and pineapples from one community's gardens for the other's forest products like fish, insects, and game. This exchange signifies recognition of mutual interdependence between Bora communities, as well as between the human and forest communities.

The remaining regions of intact forest often overlap with areas used and claimed by indigenous communities. The term "indigenous" is used here to refer to long-term[3] residents of a given place. In parts of Africa, Europe, Asia, the Arctic, and the Pacific, the majority population is indigenous, and different indigenous communities from those now present may have used forests in the past. In the Americas, indigenous forest-dwelling communities are usually marginalized, minority native Americans who are remnants of the original indigenous groups that survived conquest by Europeans and subsequent marginalization by neocolonial states. In Latin America, there are also indigenous peasant communities of mixed ancestry that rely on forests and have developed methods for managing them, often by learning from their indigenous neighbors or relatives. If it were possible for a satellite image or aerial photo to show not only the forest structure at a given place but also the tenure structure and the community organizational structures that exist in forested areas, then we would have a much better picture of the world's forests. If on top of that we could overlay the past and present forest users (including indigenous and outsider users, and the final end-users in other countries) as well as the de jure and de facto tenurial rights exercised over the areas in question, and the status of common property management systems

[3] The exact definition of long-term depends on the context and the classes of residents being compared. In many situations, long-term could reasonably be defined as at least two generations, approximately 40 years. In other cases, where conflict between a new group and an older group that has been resident for centuries is long-standing (lasting for generations), the definition of indigenous would not be applied to the interlopers whose rights to the resources have been contested for generations.

in place, then we would have an even better starting point for understanding forest dynamics and the factors behind the loss of biodiversity. But such overlays do not exist, and analyses of forest depletion are often based on incomplete information about forest users.

Indigenous residents in forested areas use the forest and its products for multiple purposes. For example, the Mexican Huastec Maya name nearly 90% of plants available in their environment (Alcorn, 1984). Two-thirds of those plants (forest and non-forest plants) have uses. While you might assume that people just go out and look for a plant when they want to use it, find it, bring it home, and use it, forest-dwelling communities manage their use of ecosystems to ensure that a wide variety of useful species are available. In communal lands owned by the Huastec, one-third of available plant species are "managed for"—in the sense that people plant or protect those species in order to maintain them within the habitats created by their general agricultural and forestry management practices (Alcorn, 1981). In the Huastec case, about one-third of available plant species are found in the forest, and 90% of forest species have specific uses (Alcorn, 1983). This level of plant knowledge and interest in maintaining useful species is generally typical of indigenous forest dwellers around the world (cf. Balée, 1994; Conklin, 1954; Messerschmidt, 1993; Warner, 1991).

Forests provide food in the form of fruits, river products including fish and reptiles, and game that can be shot in the forest or trapped in gardens scattered in the forest. Forests provide mushrooms, and edible greens and roots. Studies of forest dwellers around the world show that it is common for forest foods to make up between 50% and 80% of nutritional intake, with the higher percentages going to women, children, and poor people (Scoones et al., 1992). Certain forest leaves and roots can be burned together to produce ash that is used for salt. Forests also provide forage for forest dwellers' animals and for the animals of pastoralists that move through forested areas.

Beyond food, forest plants have numerous other uses. Plants with flowers that we find beautiful or interesting for evolutionary insights provide homes for forest people. The forest itself serves as backyards and playgrounds for children, and as workplaces for their parents. Forest plants are used to make the skeleton of a house, to lash house parts together, to thatch roofs, and to construct walls. Forest plants provide fibers used for producing household items like bags, carrying baskets, and bark cloth. Bark cloth still has ritual importance in many forested areas of the world. Large forest trees are used to make drums for communication and canoes, which serve for communication and transport. Forest dwellers generally use fresh plant and animal materials as medicine. When someone is ill, they go into their forests and gardens to collect and process fresh material daily for as long as it is necessary. The medicine is available if the habitat in which it grows has been preserved.

Local communities generally value forests beyond the products that are immediately harvested: They value forests for serving as living factories from which products can be harvested, for the natural regenerative processes that subsidize agriculture, and for their ecological function. Forest dwellers incorporate the forest into their land use systems. Swidden agriculture, also known as slash and burn, was long vilified as destruction of the forest by those who think of forest as a permanent group of standing trees. This attitude is changing as people realize that forests are really a process, a community in flux, not a permanent object (Warner, 1991). Traditional slash-and-burn farming is based on the use of forest processes (Alcorn, 1989). It begins by slashing and burning, but it depends on forest regeneration. Opening up a gap in the forest is just one small step in swidden farming. Forest dwellers often use long fallow systems that rely on natural regeneration, which is regulated at different rates across the original cleared plot. For example, among the Bora, the original opening in the forest that was planted the first year often begins regeneration in some but not all areas during the first year (Denevan et al., 1984). After several years, secondary forest covers all but the central area, which is left as a garden or agroforestry plot that contains planted fruit trees, as well as native species that came up after clearing or were protected during clearing. These kinds of complex agroforestry systems are built into swidden agriculture systems around the world (Alcorn, 1990; Messerschmidt, 1993; Olofson, 1983; Shepherd, 1992; Warner, 1991). Over generations, forest peoples often create complex anthropogenic forests that contain higher than expected numbers of useful native species, as well as introduced species.

Communities are aware of their dependence on the forest; they have altered land use and livelihood strategies in order to retain forests using methods including intensification of agriculture on non-forest lands, increased reliance on income from outside jobs, and dedication of more land to natural forests. Another common option is to go to a short fallow swidden system in order to set aside some permanent forest. In the short fallow system, fields

are fallowed for only three to five years. Native trees are protected when the fallow is slashed. Planting is done among pollarded and protected trees, and rapid secondary succession is encouraged. In these systems, agricultural fields are managed so that firewood can be harvested from native, fast-growing trees that coppice. In other cases, forest dwellers opt to intensify agriculture in permanent fields, sometimes by investing labor in creating terraces. Intensification enables them to retain, enrich, and even expand permanent forests (Padoch, 1993).

Forest products are not just managed for local use. Medicinal plants and trees that produce marketable fruits, resins, and other products are managed for sale or trade into local or regional markets. Animal skins and meat are sold to buyers who come seeking products for outside markets. Orchids and other plants are sold for ornamental uses in urban settings. High demand for such nontimber forest products can severely degrade forests, especially in cases where communities are denied the right to police extraction from their resource base.

The level of exploitation by outsiders without a long-term dependence on the local resource is often of another magnitude of impact and highly unsustainable. Sometimes outsiders just gather bags of medicinal plant samples for phytochemical and pharmacological testing for the international market. If the medicinal plant becomes commercially valuable, however, laborers hired by collecting firms can severely reduce the populations of that species to the point where local people no longer have access to the medicine (Cunningham & Mbenkum, 1993). More often, outsiders come into forests and claim the land, remove the forest, and create orange groves, plantations, or pastures. Degraded natural forests often have the potential for regeneration, but after conversion to plantation or pasture, the levels of biodiversity are generally low and regeneration of forest is unlikely.

Community-based logging for local use has had minimal impacts; community-based logging for profit also has the potential to be sustainable in communities that are concerned about long-term productivity and have strong tenurial rights over the resource (Bray, 1991). Logging by state-sponsored concessionaires, on the other hand, is usually carried out with minimal concern for long-term productivity and leaves the land with open access for settlement after the original tenurial rights have been broken. Such logging often continues even after public outcry against ecological degradation and the loss of biodiversity. In Thailand, for example, a logging ban was enacted after widespread community protests against overlogging by government-sponsored concessionaires in community forests (Lohmann, 1991). Yet logging continues in Thailand. Logs are taken into Burma and stamped as Burmese. They are then returned across the border into Thailand and sold as imported logs, since it is illegal to log in Thailand. The Thai military elite also finance and carry out logging in Burma, Cambodia, and Laos to profit from Thai market demand. In Asian and Central African forests, logging is usually done by powerful interests with links to government and the military.

Radical land use change accompanied by severe depletion of biodiversity is the type of land use against which we must compare the land use of marginalized indigenous communities that live in relatively biodiverse areas. Plantations of fast-growing pulpwood trees, rubber, oil palm and fruit, industrial logging operations, cattle ranches, and urban sprawl have rapidly transformed massive swathes of the biosphere from rich reservoirs of valuable species diversity into empty banks of weeds and crops. For thousands of years, indigenous communities have used and modified biotic communities through their land use practices, but they are not responsible for the biodiversity crisis that faces the world today.

Conflicts over Property Rights to Forests

The general patterns of forest use are similar around the world, and there are two general classes: (1) use by local communities; and (2) use by commercially oriented outsiders.

Communities rely on local forests for food, medicine, construction materials, craft materials, fuel, insurance, and products for sale/trade—in other words, a significant portion of their livelihood. Yet these uses and the rights to these forests often go unrecognized by outsiders. Local user communities often respond to reduced availability of needed forest goods and services by attempting to manage the resource for long-term sustainability if they have the tenurial rights to make decisions to control forest and land use.

Commercial operations extract the same products used by local people, but they generally exploit them for short-term profits without concern for long-term sustainability. The commercial harvest of these products, particularly extraction of timber and nontimber products by large companies, increasingly leads to depletion of forests. Extraction of commercially valuable medicinal plants has also led to depletion or local extinction of populations of those species (Lewington, 1993).

A third user class has recently been asserting its rights. Global uses of forest ecosystems have recently been recognized for achieving conservation of biodiversity and for carbon sequestration to mitigate against global climate change. The global community is increasingly claiming rights to manage forests for these uses. At a 1992 conference in Washington, D.C. (Doyle & Schelhas, 1993), North American biologists suggested that they should write "owners' manuals" for tropical forests.

As the paragraphs above make clear, forests and forest species are useful. But who owns these forests and who benefits from using and conserving this biodiversity? Forest use has led to conflicts over rights to make decisions about forest use and management, rights we may gloss as forest "ownership." While we may think of "ownership" as the "right to sell something," tenurial specialists refer to ownership or tenure as a bundle of rights and obligations as recognized or distributed by some authority (e.g., local authorities or the state). Tenure defines relationships between people and mediates their use of natural resources (Crocombe, 1971); tenure does not define relationships between people and property. Those with tenurial rights have a specific relationship to others in terms of "who" can (and cannot) "do what" and "under what circumstances" with the property in question.

Local conflicts over resources have led to the evolution of local tenure systems and supportive social mores appropriate to the culture and sociopolitical organization of the community (Berkes, 1989). What are some of the characteristics of these local tenure systems? And why are they resource conservative? Traditional tenure systems are in effect a partnership between individuals and the community to maintain a community's resource base by limiting access and imposing restrictions on forest use. Traditional tenure systems are extremely variable, complex mixtures of private and community rights (Crocombe, 1971). It is not simply that community members share joint ownership; rather, different community members have different rights and obligations. And different kinds of rights are exercised over different kinds of resources. Agricultural lands, for example, are often held by individual families, while rights to forest or pasture lands are more likely to overlap. These overlapping rights result in a sharing of benefits across a broad range of the community. Claims to a given patch of forest or even particular trees may be held by several different people, or different groups of people, at different times of the year, for example. These overlapping claims work to exclude non-community members and protect the community from easy acquisition by outsiders, or from exclusive use by any individual who might destroy the community's forest resource base.

Customary tenure systems vary widely from place to place, yet they are similar in that they derive their legitimacy from the community's authority. Resource rights are inherited in complex ways, and bundles of rights often get reorganized in each new generation. Customary rights and obligations evolve with the changing availability of the resource, changing demands for the resource, and changing relationships between user communities. If forests are under stress, local regulations are often established and then enforced by local forest protection committees. The effectiveness of these common property systems depends on widespread acceptance and adherence to rules governing access, strong local institutions that administer local justice, and guidance by local leaders committed to the values of the system. Some have described traditional tenurial systems as a form of "institutional capital" (Field, 1984), because compliance is sustained with low investment on enforcement.

Community institutions, authority, and rights to forests, however, are almost invariably ignored when decisions about forests are made at the national level or in the context of colonial expansion. Such national-level decisions lead to conflicts both between the state and the community and within and between communities. These conflicts are very old. For example, in the late 1500s, Guatemalan communities argued in Spanish colonial courts that their forests should not be given over to Spaniards for conversion to pasture, because these forests belonged to them and were an integral part of their productive base (M. J. MacLeod, pers. comm., 1992). The same arguments can be found in the historical literature from Europe (Westoby, 1989), Africa (Porter et al., 1991), and Asia (Scott, 1976). Today, communities make the same arguments in the face of forest alienation for state-sanctioned logging, mining and petroleum ventures, plantations, and colonization or resettlement programs. In 1993, the Embera, Kuna, and Wauna communities in the Darién region of Panama mapped their forests in an effort to register their rights to ancestral lands in the face of settler encroachment and the threatened completion of the last segment of the Panamerican Highway (Denniston, 1994). The forest protection committees established by communities to exclude their neighbors and regulate community forest use are usually unable to keep out powerful outsiders.

The historical trend is similar around the world.

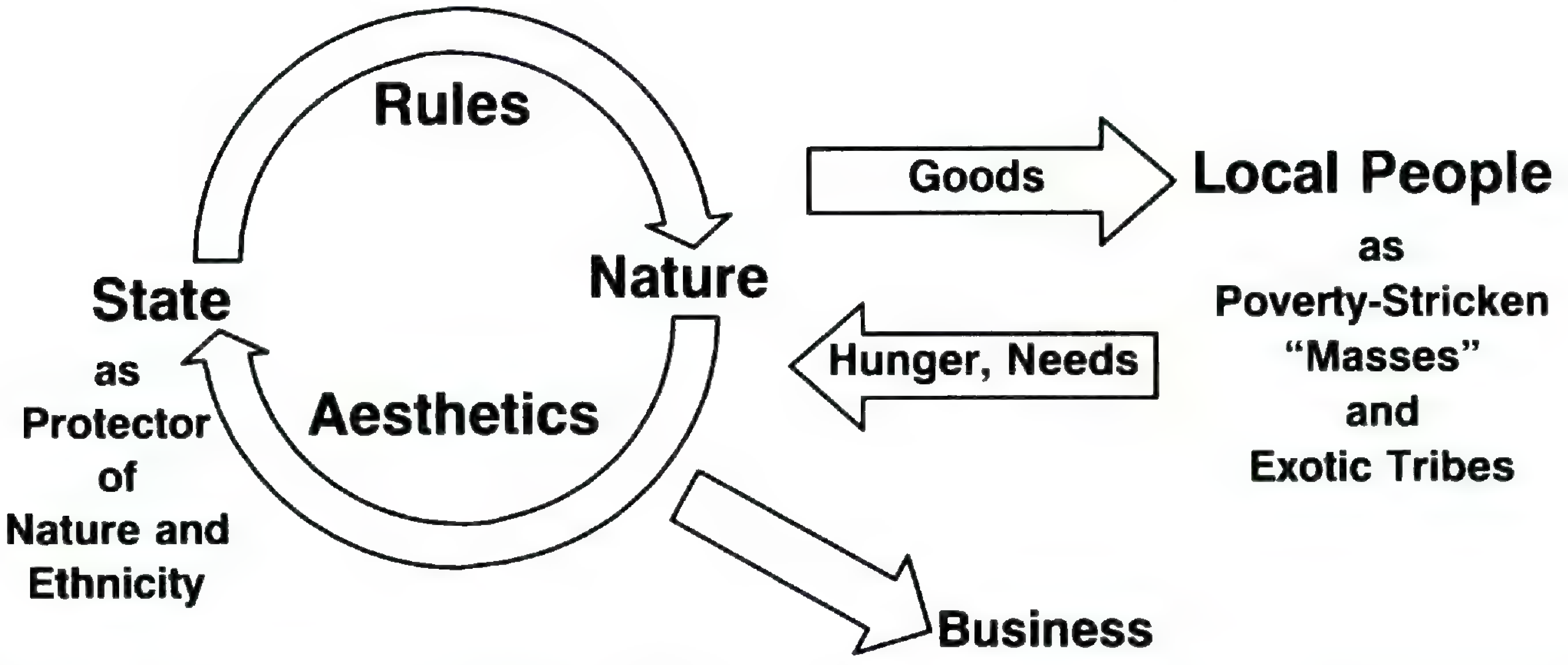

FIGURE 1. Analysts' standard frame for conservation discourse. This model is inadequate for depicting causative relationships or generating viable solutions.

Initially, despite the fact that forests and adjacent agricultural lands were being administered under traditional tenurial systems, named in local languages, and claimed by local peoples, colonial authorities and/or the neocolonial state limited communities to certain specified forest product "usufruct rights" which were then downgraded to "privileges." Guha (1983) lucidly described this process in India, for example. Eventually, states deny resident communities any rights or privileges in the state's forest, except perhaps temporary employment as laborers. In other cases, the forest is turned directly over to government-sanctioned concessionaires, ranchers, or plantation companies; and communities immediately lose rights to their forest and become labeled "squatters" instead of being recognized as holders of any property rights at all. When community authority was usurped by the state, community property became transformed into "no one's property," no one took responsibility for long-term forest management, and forest degradation ensued. Hardin's (1968) famous "Tragedy of the Commons" model was really about the tragedy of "open access" (Berkes et al., 1989, and others). Many forests were well managed under common property regimes, but they were transformed into open access forests after logging or development projects ignored, and thereby invalidated, local systems of forest control. Once under open access, their biodiversity has been depleted by all and sundry, locals and outsiders.

Analytical Frameworks

In summary, plants are useful to people who live in biodiverse areas. Useful plants are managed by people so the plants are sustainable. People have developed complex agricultural systems that incorporate wild plants, and communities have developed their own systems of tenure to govern the extraction of plants under common property regimes. But common property regimes have been overridden by states who have claimed rights over those resources.

How does this information fit into the ways we commonly think about depletion and conservation of biodiversity? Figure 1 shows the standard conservation discourse frame—the way that people usually talk about biodiversity loss and plan what can be done to stop it. The major players are identified as the state, nature, and the hungry, growing population. The state is envisioned as the protector of nature through enforcement of rules in order to preserve nature for its aesthetic values. The state enacts regulations and sets aside land for national parks, reserves, and wildlife sanctuaries. Rural people are portrayed as poverty-stricken masses that are hungry and needy. They have no apparent organizations or local institutions. Their communities' sociopolitical structures are invisible. As their numbers increase, or their cash needs increase, the model predicts that rural masses will extract more and more goods until nature is destroyed. If forest dwellers are included in the analysis, the standard discourse frames them as exotic tribes having aesthetic value in their "ethnic" customs and colorful costumes whose aesthetic ethnicity should be protected. Business, if it is mentioned, is seen as a minor player. Corruption of state officials in performing their responsibilities is sometimes raised as an issue, but rural people living near or in nature reserves receive the bulk of the

FIGURE 2. Grassroots support groups' standard frame for conservation discourse. The "balance with nature" is not necessarily stable.

blame for forest destruction under this standard frame of discourse.

Figure 2 shows an alternative that is increasingly used by environmental nongovernmental organizations (NGOs), particularly national-level NGOs in developing countries. This frame of discourse is based on a focus on nature conservation at the local level, and is very old among peoples indigenous to a particular locality. In this circle, nature and community are part of one whole. Products and processes are used by the community, but the community's rules govern how nature is appropriated. Feedback from poor management practices is recognized locally, and new controls are enacted to modify use so the ecosystem can rebound. In this circle, nature is treated as a power just as we treat the state: nature passes judgment and nature "sets the table" for negotiation of what is acceptable and unacceptable behavior among people.

A marker for this kind of frame of discourse is often the use of words like "harmony with nature" or "balance with nature," but it is probably better to think of this circle as a dynamic relationship in which the balance shifts back and forth over time. This relationship does result in extinction of some local populations of plants and animals, especially large mammals, but generally does not significantly lower overall local levels of biodiversity.

In this circle, poems, stories, proverbs, pictures, and local histories reinforce and maintain people's relationships with each other and nature. Local space is seen as containing history and ancestors. Many forest dwellers around the world say something loosely translated into English as, "the ancestors will punish us if we do not take care of what they have given us." The nature-community circle is the key to personal identity. As one man in the Solomon Islands said (J. Cordell, pers. comm., 1991), "I couldn't sell my land to you, it would be like cutting off my arm and selling it to you. It would be of no use to you." Parting with his land would destroy him, and it was inconceivable to him that anyone could use that land and its resources without being part of his ancestral lineage and part of his community. Indigenous Amazonian societies, for example, generally conceive of their territory as having many vertical dimensions from the air down to the forest, the land, the water, and the underground. When Colombian indigenous communities were recently awarded tenurial rights to reserves for which they had been fighting, they were startled to learn that the reserves only gave them rights to the surface land, and not the forest, the water, the air, and the underground resources. They were upset at what had been taken away from them. They had not imagined that territory could be carved up in that way (E. Reichel, pers. comm., 1992).

As Figure 3 illustrates, at the local level human communities adapt differently from their neighbors, and their impacts on the same environment differ according to their culture, their community organizations, and their histories. Communities often develop institutional mechanisms for excluding neighboring communities from appropriating their resources. Warfare and trade form the borders between neighboring circles, but the borders are flexible and shift with time. Trading networks lead to exchange of resources between circles. This model is adequate to describe most relationships prior to the colonial era and subsequent expansion of the global economy.

Over the past 500 years, however, the situation has changed to one more like that shown in Figure 4. This diagram illustrates the economic development process from a global perspective. On the right are the local circles from the previous figures. On the left is the state-based Eurocentric elite community of which we are a part. It includes the "developed countries" as well as the elites of "developing countries." The elites of the world's states form a community with mutual financial interests. From colonial times to the present, the Eurocentric community has acted under market ethics and used violence, speed (sensu Virilio, 1978), and products to extract capital, information, raw materials, and

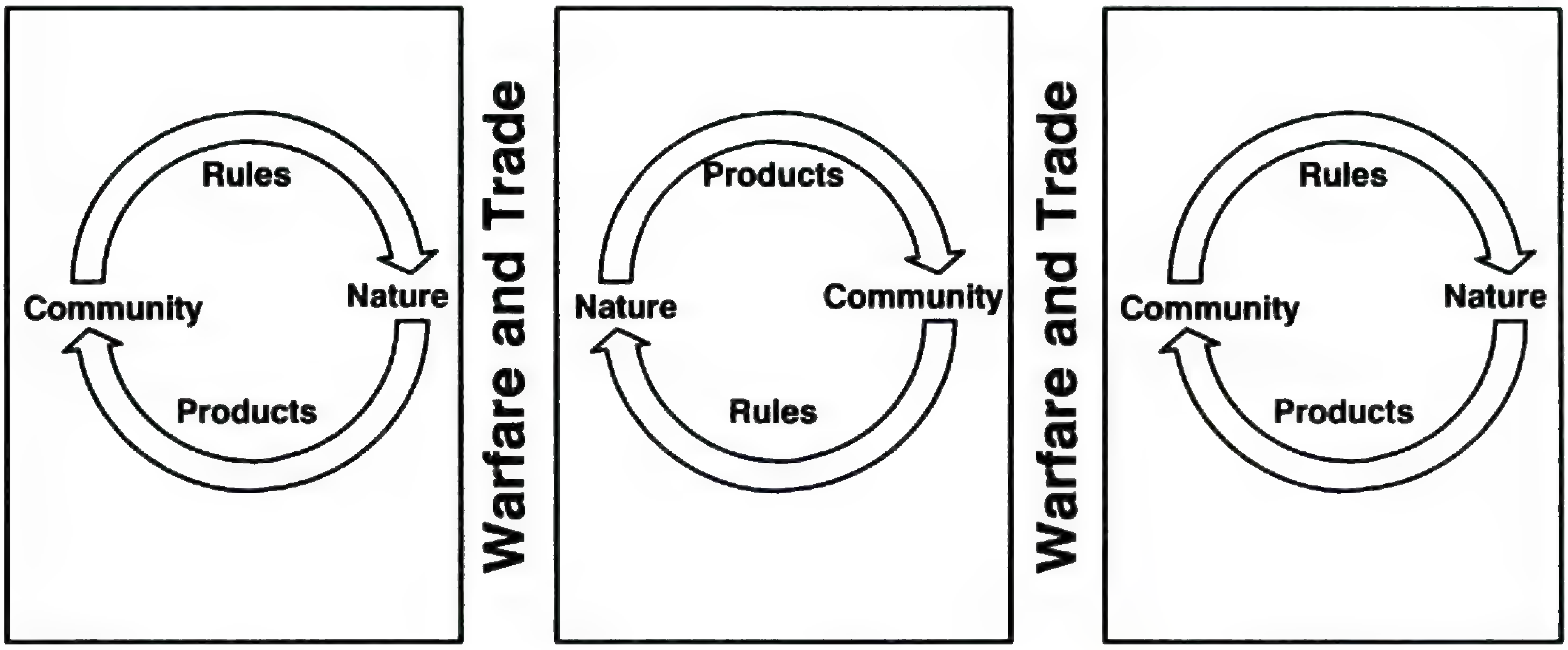

FIGURE 3. Model of global nature-community relationships prior to 1500. This model emphasizes the role of warfare and trade that influenced local use and management of nature.

labor from the local, culturally and biologically diverse nature-community circles around the world. Economic botanists participated in the extraction of information and genetic resources. Under market ethics, the market is allowed to be the primary allocator of resources without consideration for social or environmental impacts of that allocation.

Europeans and then neocolonial states have followed a process of breaking the community (as in the broken top and bottom circles in Fig. 4). They broke the authority of local organizations, local feedback processes, and local rules and ethics in order to extract resources. Entrance into market economies weakens local cohesion as a few entrepreneurs emerge and claim individual rights over what had been communal resources. Many communities evolve new mechanisms to maintain resources after the entrance into the market economy, but this often depends on communal rights to control and allocate resources. This small-scale unraveling must be seen against the larger context of state-level direct or indirect usurpation of local resources. Indirectly, state-sponsored individual titling of land previously held by communities had

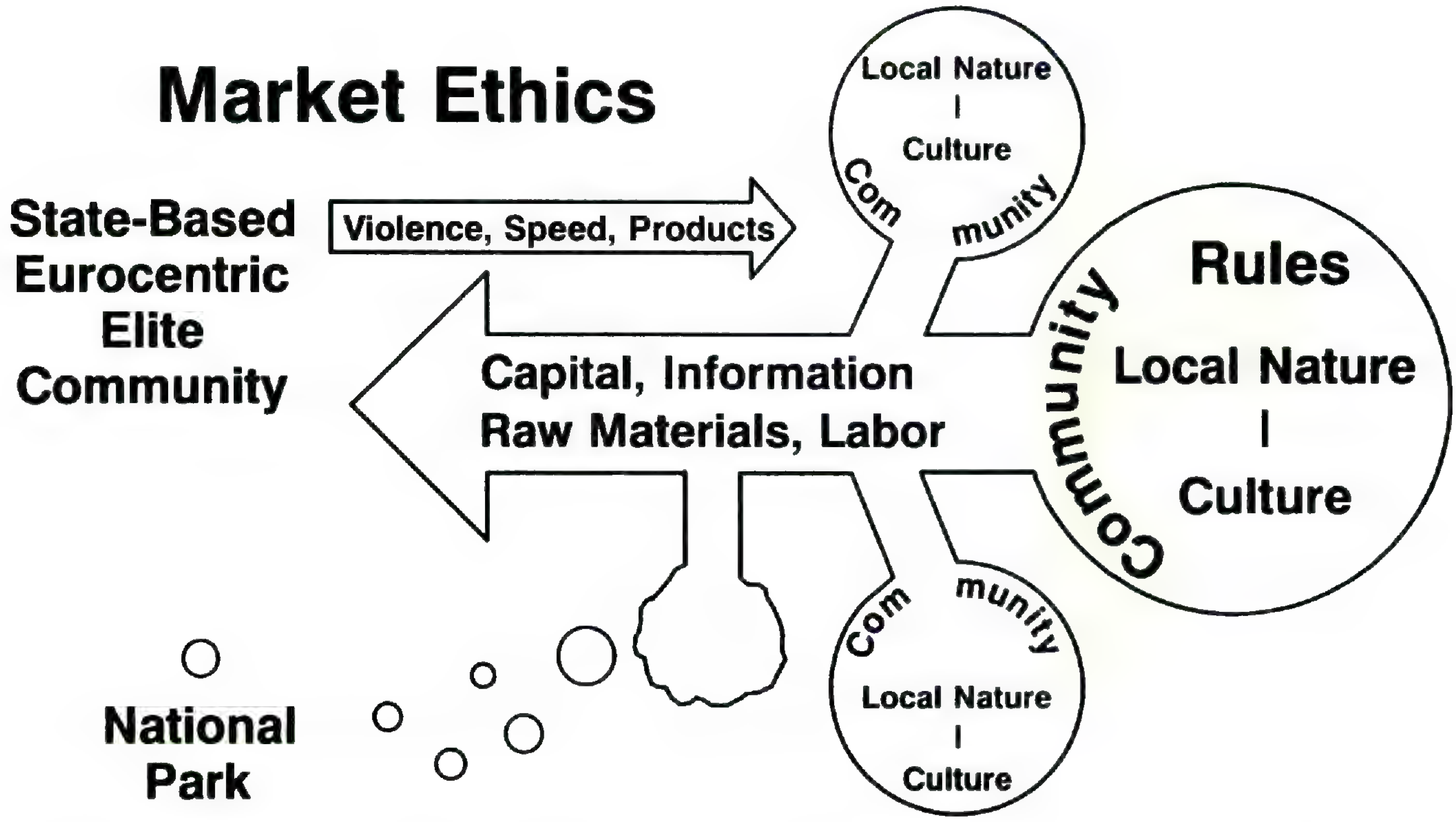

FIGURE 4. Model of global nature-community relationships during the colonial and neocolonial periods. This model emphasizes the destructive extraction of biodiversity.

often led to increased resource degradation and loss of forest. The state usually awards title to only one of those who have claims on the property in question in order to bring the resource into the capitalist system as a commodity. Those seeking titles are often entrepreneurs who then sell the forest to outsiders who have no long-term interest in maintaining the forest. In other cases, indigenous community lands are actively reorganized by development programs that award individual holdings to current residents and add settlers to the community mix, a practice that has had negative impacts on forest and other natural resources (Porter et al., 1991).

Historically, most states have directly usurped forests from local communities by assuming state's rights over forests in order to increase their tax base and export revenues from crops; they have extracted resources directly or promoted the conversion of forests to agricultural lands. In many places, states are executive committees of elites who make policies and laws enabling themselves to use their powers for their own personal benefit, not for the public good. States often claim authority to replant or manage forests, but they seldom take full responsibility for that obligation. Few states are held publicly accountable for meeting their obligations, and their forests continue to shrink under de facto open access conditions. In addition to direct state-sponsored deforestation, in many countries there is what has been called "institutional legitimization of deforestation" (G. Murray, 1988, unpublished). For example, titles are often only granted to deforested land, because by deforestation the owner demonstrates that he will put the land to a use that will generate tax revenue for the state. Settlers are often encouraged to move into lands claimed by indigenous peoples. The state's failure to recognize community rights to forests, and carry out its obligations to defend community rights in situations where those rights have been recognized, has contributed to the loss of biodiversity shown in Figure 4. The ultimate result of this process is that communities are broken, and their ability and commitment to maintaining their local natural base is weakened. This is shown in the diagram as local circles of biodiversity shrinking and disappearing (right to left). Communities attempt to adapt to the above stresses while maintaining biodiversity, but their levels of "good enough" biodiversity are down-graded as they accede to the shrinking process depicted in the diagram (Lynch & Alcorn, in press).

On our side of this equation, on the left, we do not realize the effects of our choices about what we buy. We do not see how what we buy affects environments and peoples far away from us. There is no feedback loop to warn us that we are damaging their local natural resource base, yet, according to Paul Erhlich (1993), the richest 20% of the world's population (we Eurocentric elites) are responsible for 70% of the destruction of the world's environment. At the national level, as nature shrinks in the landscape, states are encouraged by conservation interests to establish national parks and reserves, which we know are essential but at best only protect a small bit of biodiversity and are often only paper parks. On the far righthand side of the diagram is the intact community blocking the extraction process and protecting local nature. Resistant circles like this one are usually found in remote areas, in the forests of the Philippines, the Amazon, Papua New Guinea, Zaire, and Panama, for example. On a global scale, there are fewer and fewer of such circles left.

This resistant community takes many forms. While it might initially appear that this circle is the same as the earlier local nature-community circles, it is not. Communities in these circles have often engaged in various acts of resistance over the past centuries without any outside help. Today, however, resistant communities are often supported by a coalition that includes some elements of the Eurocentric elites. Sometimes that assistance includes publicity about struggles over rights to biological resources. In Asia, Africa, and Latin America, desperate people continue to risk losing their lives by standing up before the most powerful elements of their governments and local power structures in order to save forests upon which they depend for livelihood and identity. The story of Chico Mendez's murder was made famous by international conservation organizations, but he is one of thousands who have died defending their rights to tropical forests.

Figure 5 shows the addition of the bottom arrow signifying supportive input under conservation ethics. The feedback and authority in the resistant circle remain local, but outsiders bring in capital, speed, information, and market linkages to support the local community in maintaining significant control over its piece of nature, in effect forming partnerships. This includes partnerships with the state. The role of the state can be negative, as I have described, but the state has an essential positive role to play in legitimizing and defending communities that are trying to manage their forests. That includes recognizing and defending communal tenurial rights, recognizing rights to forests and other economically valuable biodiversity, and en-

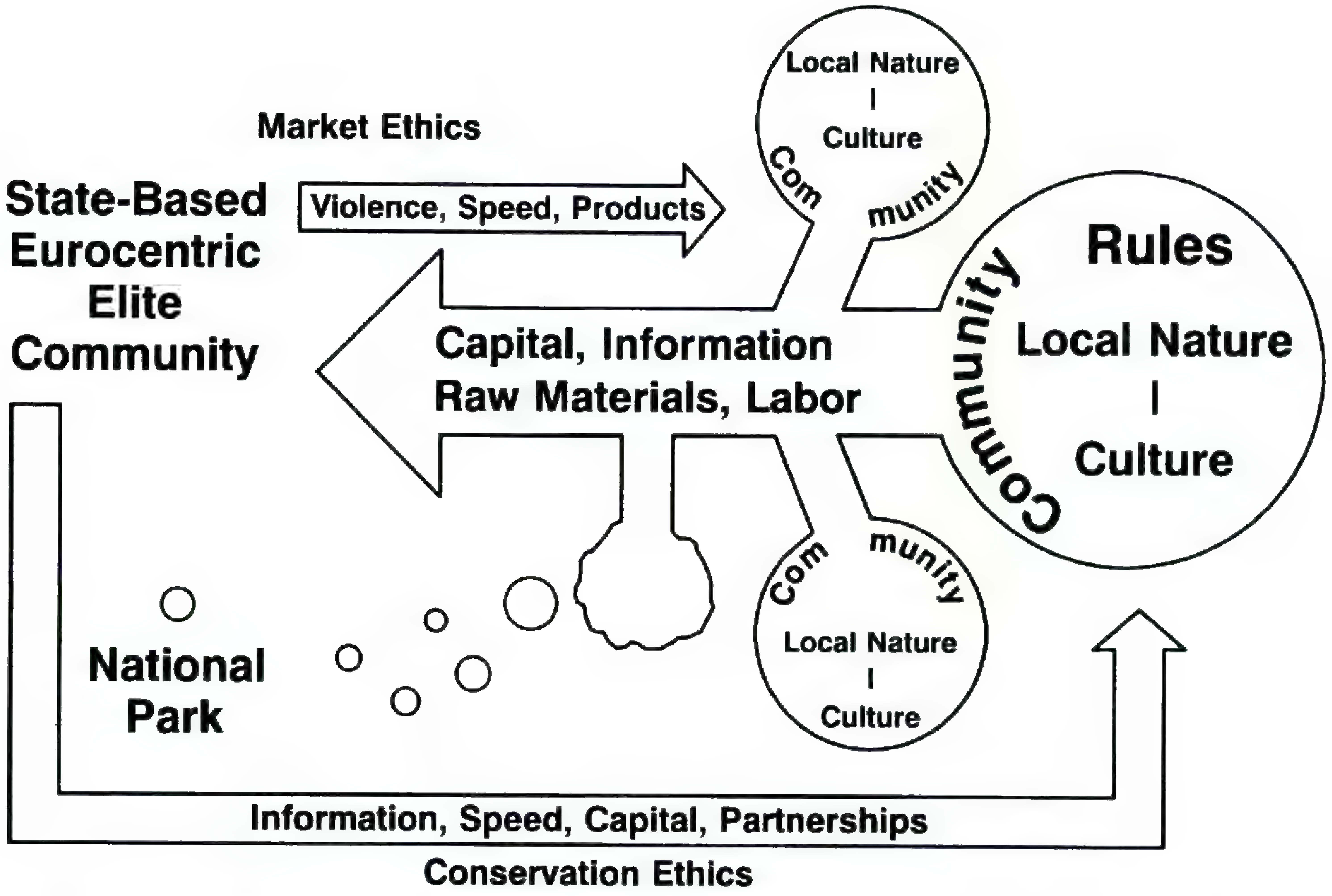

FIGURE 5. Alternative model of global nature-community relationships. This model offers a revised frame for conservation discourse.

suring that benefits from intellectual property rights are distributed to communities that live where useful biodiversity is found. India and Nepal, for example, have pioneered some unique forest "co-management" partnerships appropriate for South Asia. Another option is found in the forest leases being implemented and considered in Indonesia, the Philippines, and Thailand (Fox, 1993). In the Philippines, the state has prevented migrant farmers from entering forests that are formally leased to indigenous peoples. Other options include the extractive reserves and indigenous reserves of Latin America. Mexico's ejidos and comunidades provide communal tenure and a haven for biodiversity (Toledo, 1992). Fully 70% of Mexico's forest cover is on communally owned lands (Bray, 1991), but those forests are now in jeopardy under new Mexican privatization policies being enacted in support of the North American Free Trade Agreement (NAFTA).

The New Connection to Economic Botany

What does this new trend of partnerships under conservation ethics have to do with economic botanists? What is the new connection? Economic botanists are now working to link conservation, development, and respect for local rights. Various professional biological societies, including the Society for Economic Botany, have drafted ethical guidelines to encourage members to respect local rights. The World Wide Fund for Nature (Cunningham, 1993) recently published a review of ongoing initiatives, the issues, and alternatives for creating equitable partnerships in new natural products development.

The most high-profile and controversial effort to address these issues is the promotion of intellectual property rights to plants or knowledge about plant uses. Intellectual property rights, or IPR, has appeal, because it attempts to address equity issues and recognize the rights of disenfranchised groups. On the other hand, there are many questions about whether IPR is the appropriate tool for achieving these objectives (Axt et al., 1993). There is little evidence that states will be able to effectively distribute royalties back to the rural communities that were the original source of the plant or the knowledge. Given states' past behavior, it is unlikely that benefits will be shared with marginal groups or used to support conservation in most countries. There are larger questions about whether species or knowledge about them should be owned by anyone. Representatives from some indigenous peoples' or-

ganizations have stated that no one should own knowledge or species because they are part of God's creation. They further state that no one has the right to profit from knowledge that could be shared for the benefit of all. They are acting on their traditional values that support sharing benefits across the community. Many other indigenous groups, however, have gone on record declaring their intellectual property rights and demanding international protection for those rights (U.N. Working Group on Indigenous Populations, 1992). These groups recognize that their knowledge and plants are generating large profits for others, and those others are not sharing the profits. But the concept of collective rights is not well developed in Western law, which has focused on rights of the individual vis-à-vis the state (Axt et al., 1993).

For many, the issue of intellectual property rights is inseparable from the issue of land rights, which have been ignored for centuries. Both issues are seen as the reflection of the larger issue of human rights abuse against marginal communities. At this time, however, land rights and intellectual property rights are not being linked by international conventions, and it is not clear that developing royalty agreements will contribute to conservation or bring benefits to local communities.

In addition to their involvement with IPR agreements, economic botanists are also involved in on-the-ground efforts to establish or provide assistance to green enterprises that will empower local communities to enter this system in ways that are advantageous to those communities. Useful plants form the basis of many fledgling green enterprises today. Economic botanists are also providing assistance with surveys, rapid information exchange networks, and databases. These efforts are useful if they ensure that communities and grassroots nongovernmental organizations, not just the elite, have access to the information about useful plants, their markets, market dynamics, and prices.

Partnerships are being formed between researchers from universities and nongovernmental organizations to promote grassroots development and conservation. Partnerships between universities and local communities are also proving especially helpful. The prestige and profile of academic institutions alone can help politically to strengthen the state's commitment to defending communities' tenurial rights. The Amazon Program of the Woods Hole Research Center and its Brazilian collaborating organizations (including EMBRAPA, several universities, and others) is an outstanding example of a program promoting information sharing between researchers, government agencies, and organizations representing rural communities in order to conserve forests (Nepstad & Schwartzman, 1992). The New York Botanical Garden's Institute of Economic Botany has forged innovative partnerships with the Belize Association of Traditional Healers, the Ix Chel Tropical Resource Foundation, Grinnell College, the Belize Center for Environmental Studies, and the Belize Forestry Department. Together they have created an ethnobiomedical extractive reserve (Balick et al., 1994).

Private sector companies that benefit from biodiversity and traditional ethnobotanical knowledge are also seeking ways to support communities in addition to direct profit sharing. For example, Shaman Pharmaceuticals founded The Healing Forest Conservancy to strengthen communities and conserve "biocultural diversity" through promoting sustainable harvest of natural products, enhancing opportunities for communication between forest societies and urban-based societies, and encouraging policymakers to develop initiatives that support indigenous institutions and foster the improved welfare of indigenous peoples (Anonymous, 1994).

Ecologists and botanists are helping in other ways. For example, some communities are seeking external assistance from scientists to ensure natural forest regeneration, after they have realized that decisions to market forest products are beginning to deplete their resource base. In other cases, communities need the help of ecologists or forestry specialists to certify that their community forests are not "degraded" or economically useless in order to disprove state claims that the useless condition of the forest warrants reforestation by concessionaires. The state often directly or indirectly funds concessionaires to cut down community forests in order to create plantations. This deprives communities of access to the many forest products on which they currently depend and repeats the historical process of human rights abuse and biodiversity depletion outlined earlier. When biologists provide officials and communities with information about the health of natural forests, the efficiency of natural regeneration for renewing forests, and the values of natural forest versus plantation, both biodiversity and local communities benefit.

Conservation organizations are expanding from their traditional focus on state management of national parks and reserves to support "integrated conservation and development" projects, known in conservationists' jargon as ICDPs or ICADs, that often involve co-management partnerships between the state and neighboring communities to support conservation and sustainable use of biotic resources

for the economic benefit of local communities (Brown & Wyckoff-Baird, 1992; Wright et al., in press).

Yet, in keeping with the standard frame of discourse in Figure 1, many people say, "But once 'these primitive people' get a little money they will destroy their natural environment in order to buy things." And they ask if community-based conservation requires restriction on development. My response is to note that no rural people are living in pristine conditions today. The people who are struggling to maintain their forests also use money, have plastic buckets, and wear polyester clothing. They use deodorant, and some of them have televisions, motorcycles, motorboats, and cars. They have been touched by the cash economy and by development. They interact with the market. Yet, they are concerned about the condition of their forests and are taking measures to manage them. They show us that development does not necessarily mean that people will no longer depend on their natural resources and consequently be expected to destroy their forests. Certainly as development proceeds, some communities abandon long-term concerns in favor of short-term gains, particularly when their tenurial rights are weak, but other communities do not (Alcorn, in press; Lynch & Alcorn, in press).

Figure 5 offers a more sophisticated frame of discourse for recognizing, communicating, and assessing the complex processes that threaten states' and rural peoples' efforts to conserve biodiversity—efforts that are invisible to those using the frame of discourse diagrammed in Figure 1. I encourage those whose immediate reaction is that local communities do not and/or will not conserve biodiversity to reconsider the situation using an alternative analytical framework including the factors raised in my discussion of Figure 5.

If one accepts the premise that local communities can and will conserve biodiversity, and works within the framework in Figure 5, the challenge becomes one of finding ways to identify and support local initiatives to link economic development with biodiversity conservation, traditional systems of knowledge, and local institutions including common property resource management systems (Alcorn, 1991; Gadgil et al., 1993; Seymour, in press). Conservationists and communities are increasingly joining in innovative partnerships and seeking the help of botanists in order to achieve local conservation and development goals. There is hope that more and more economic botanists will become aware of their relationships to the loss of biodiversity and use their expertise to play a positive role in supporting equitable development and the conservation of biodiversity.

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PHARMACEUTICAL PROSPECTING AND THE POTENTIAL FOR PHARMACEUTICAL CROPS. NATURAL PRODUCT DRUG DISCOVERY AND DEVELOPMENT AT THE UNITED STATES NATIONAL CANCER INSTITUTE[1]

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ABSTRACT

Chemically-complex natural product drugs are not readily synthesized, and large-scale production for clinical and commercial development often involves isolation from the natural source. The rapidly escalating demand for taxol, originally isolated from the bark of *Taxus brevifolia*, has emphasized the need for alternative sources to the wild plant, and the National Cancer Institute (NCI) has developed policies for exploring such sources at the early stages of preclinical development of potential new drugs. The potential for pharmaceutical crop development in the case of several possible anti-AIDS agents will be discussed.

Humans throughout the ages have relied on plants as a source of food, clothing and construction materials, cosmetics, and medicines. With the soaring world population now exceeding 5.5 billion (World Population Data Sheet, 1993), the consumption of plant and other natural resources is escalating at an unprecedented rate. There is an urgent need to conserve these essential resources through the development of sustainable methods of harvesting and environmentally sound cultivation practices.

Sophisticated traditional medicine systems have been in existence for thousands of years in countries such as China (Chang & But, 1986) and India (Kapoor, 1990), and in the New World tropics (King, 1992). These plant-based systems continue to play the major role in the primary health care of about 80% of the world's inhabitants (World Health Organization statistic, Farnsworth et al., 1985). It has been estimated that plant products constituted at least one active ingredient in about 25% of the prescriptions dispensed from community pharmacies in the United States from 1959 to 1982 (Farnsworth et al., 1985). Well-known examples of plant-derived medicinal agents include the antimalarial drug quinine, the analgesics codeine and morphine, the tranquilizer reserpine, and the cardioactive agent digitoxin.

Despite this evidence, pharmaceutical companies in the western, developed world until recently showed little interest in the investigation of plants as sources of potential new drugs. In recent years, however, major companies, such as Glaxo Chemicals, Merck Pharmaceuticals, Monsanto Company, and SmithKline Beecham, have started screening plants for a range of biological activities. The plant samples are generally provided through collections carried out by botanical institutions or intermediary organizations, some of which are located in developing source countries. The development of these collection and screening operations has been accompanied by an increasing awareness of the need to conserve valuable biological resources, and a recognition of the rights

[1] NCI gratefully acknowledges the collaboration and support of the many individuals and organizations worldwide that make these programs possible. NCI recognizes the indispensable contributions being made through the provision of valuable natural resources, expertise, knowledge, and skills; through policies of collaboration and compensation, as stated in the Letter of Intent, NCI wishes to assure participating countries of its intentions to deal with them in a fair and equitable manner.

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of source country organizations and peoples to the accrual of benefits from the development of any new drugs derived from their countries' genetic resources.

The National Cancer Institute Plant Acquisition Program

Historically, many claims have been made for the beneficial use of plants in the treatment of cancer (Hartwell, 1982), though many of these claims may be viewed with some skepticism, since cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine. The development of efficacious anticancer agents, such as the so-called Vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don, and etoposide and teniposide, semisynthetically derived from podophyllotoxin isolated from *Podophyllum peltatum* L. and *P. emodii* Wall. (Cragg et al., 1993b), provided convincing evidence that plants could be sources of a variety of novel cancer chemotherapeutic agents. Prompted by such discoveries, in 1960 the National Cancer Institute (NCI) initiated a systematic program for the collection and screening of plants for antitumor activity. Between 1960 and 1981, the NCI, in collaboration with the United States Department of Agriculture (USDA), collected and tested more than 114,000 extracts of some 35,000 plants against a range of animal tumor systems (Perdue, 1976). While a large number of agents, belonging to a wide variety of chemical classes, were isolated and characterized (Hartwell, 1976; Suffness & Douros, 1979), few satisfied the stringent preclinical development requirements and advanced to human clinical trials. Clinically active agents to emerge from this program included taxol from *Taxus brevifolia* Nutt. and other *Taxus* species (Cragg et al., 1993a), topotecan and CPT-11, semisynthetic derivatives of camptothecin from *Camptotheca acuminata* Decne., and homoharringtonine from *Cephalotaxus harringtonia* var. *drupacea* (Sieb. & Zucc.) Koidzumi (Cragg et al., 1993b).

The NCI–USDA collaborative program concentrated on plant collections in temperate regions. Since 1986, however, the NCI has expanded its program to the collection of plants from tropical and subtropical regions worldwide through the award of contracts to the Missouri Botanical Garden (Africa and Madagascar), the New York Botanical Garden (Central and South America and the Caribbean), and the University of Illinois at Chicago, assisted by the Arnold Arboretum at Harvard University and the Bishop Museum in Honolulu (Southeast Asia). In carrying out these collections in over 20 countries, these NCI contractors work closely with qualified organizations in each of the source countries. Scientists from source country organizations collaborate in field-collecting activities and taxonomic identifications. Their knowledge of local species and conditions has been indispensable to the success of the NCI collection operations to date. Source country organizations provide facilities for the preparation, packaging, and shipment of the samples to the NCI natural products repository in Frederick, Maryland, and their personnel have provided invaluable assistance to the NCI contractors in obtaining the necessary collection and export permits. The collaboration between the source country organizations and the NCI collection contractors has, in turn, provided support for expanded research activities by source country scientists, and the deposition of a voucher specimen of each species collected in the national herbarium is expanding source country holdings of their own flora. In addition, NCI contractors provide training opportunities for local personnel through workshops and lectures.

In expanding the plant collection program to tropical and subtropical regions, the NCI recognized that tropical forests contain well over 50% of the estimated 250,000 plant species found on earth; only a small percentage have been investigated for their chemotherapeutic potential, and relatively few had been screened in the earlier NCI program. In addition, the rapid destruction of tropical forests and the disappearance of indigenous knowledge associated with the use of many of the plants lent an urgency to the initiation of a collection program before these valuable resources had been too severely depleted. While investigating the potential of these plants in drug discovery and development, the NCI also wishes to promote the conservation of biological diversity and recognizes the need to compensate source country organizations and peoples in the event of commercialization of a drug developed from a plant collected within their borders (Cragg et al., 1994).

Extracts of the plants are tested in vitro for selective cytotoxicity against panels of human cancer cell lines representing major disease types including leukemia, melanoma, lung, colon, breast, central nervous system, ovarian, and renal cancers (Boyd, 1989; Grever et al., 1993). With the emergence of the acquired immunodeficiency syndrome (AIDS) as a global epidemic in the 1980s, the NCI was assigned the additional mission of the discovery

and preclinical development of novel anti-HIV agents; since 1988 all plant extracts have also been tested for in vitro anti-AIDS activity in a screen comprising human lymphoblastoid cells infected with the live AIDS virus (Boyd, 1988). Extracts showing significant selective cytotoxicity or anti-AIDS activity are subjected to bioassay-guided fractionation by NCI chemists, and the isolated active agents, after complete structural characterization, are entered into various stages of preclinical development (Cragg et al., 1993b). Satisfactory completion of preclinical studies and approval by the Food and Drug Administration (FDA) clears the drug for advancement to clinical trials and, subject to further FDA approval, to eventual marketing and general clinical use.

Even though the percentage of extracts showing preliminary activity in the in vitro screens might vary between 1% and 5%, the number of potentially valuable "leads" approved for preclinical development is more likely to be one in 5000 to 10,000. Given the stringent requirements for preclinical and clinical approval, the chances of developing an effective commercial anticancer drug against any of the diseases tested for are of the order of one in 50,000 samples screened. The timespan for development can vary considerably, from 10 to 20 years for anticancer drugs, with the cost estimates for discovery and development exceeding $230 million (DiMasi et al., 1991).

Drug Development and Supply Issues: The Potential for Pharmaceutical Crops

The initial plant sample (0.3–1.0 kg) collected by the contractor generally yields enough extract (10–40 g) to permit isolation of the pure, active constituent in sufficient milligram quantity for complete structural elucidation. Subsequent secondary testing and preclinical development, however, might require gram or even kilogram quantities, while approval for clinical development could require multi-kilogram quantities.

To obtain sufficient quantities of an active agent for preclinical development, re-collections of 5 to 200 kg of dried plant material, preferably from the original collection location, might be necessary; considerably larger quantities would be required for subsequent clinical development. Large re-collections necessitate surveys of the distribution and abundance of the plant, as well as determination of the variation of drug content in different plant parts and the fluctuation of content with the season of harvesting. The potential for mass cultivation of the plant would also need to be assessed. If problems are encountered due to scarcity of the wild plant or inability to adapt it to cultivation, a search for alternative sources would be necessary. Other species of the same genus or closely related genera can be analyzed for drug content, and techniques, such as plant tissue culture, can be investigated. While total synthesis must always be considered as a potential route for bulk production of the active agent, it should be noted that the structures of most bioactive natural products are extremely complex, and laboratory bench-scale syntheses often are not readily adapted to large-scale economic production.

Of the established plant-derived commercial anticancer drugs, vinblastine and vincristine are still produced by isolation from *Catharanthus roseus* grown in various regions worldwide, while etoposide and teniposide are semisynthetically produced from natural precursors isolated from *Podophyllum emodii* harvested in India and Pakistan. Over the past 20 to 30 years extensive research has been carried out on the development of synthetic and tissue culture procedures for the production of these drugs, particularly the Vinca alkaloids, but use of the plant sources remains the most economically viable method.

The development of sources for the large-scale production of taxol illustrates the necessity for studying the various methods of biomass production at an early stage of drug development in order to cater to escalating demands (Cragg et al., 1993a). Between 1977 and 1988 supplies for preclinical and early clinical development were easily met by isolation from the bark of the Pacific Yew (*Taxus brevifolia*), the original source of the drug. Up to 1990, approximately 4 kg had been isolated from the bark, but discovery of the efficacy of taxol in the treatment of refractory ovarian cancer in 1988 increased the potential demand to over 24 kg per year in the United States alone. Given the yield at that stage of one gram of taxol from approximately 13 kg of dried bark (equivalent to about 1.5 mature trees), the anticipated demand of over 24 kg required the processing of 312,000 kg from about 36,000 mature trees.

The discovery of taxol's efficacy in the treatment of breast cancer, and observations of preliminary activity against other cancers, such as lung and head-and-neck cancer, created potential demands exceeding 400 kg per year in the United States, and it was clear that alternative sources to the bark would need to be developed. In 1991, the pharmaceutical company Bristol-Myers Squibb (BMS) signed a Cooperative Research and Development Agreement (CRADA) with the NCI whereby it be-

came responsible for the continued production of taxol. Since the bark of *T. brevifolia* remained the only FDA-approved source of taxol, BMS and its partners, Hauser Chemical Research and Hauser Northwest, continued bark collections of over 700,000 kg per year in 1991 and 1992. BMS and the NCI, however, immediately embarked on intensive studies of alternative methods of bulk production and alternative sources of taxol. A key to solving the taxol supply problem was provided by the pioneering studies of French workers, who demonstrated that natural precursors, such as baccatin III and 10-desacetylbaccatin III, can be converted to taxol and related active agents by relatively simple semisynthetic methods (Denis et al., 1988). Extensive analytical surveys of *Taxus* species and cultivars worldwide showed that the needles of many species contain reasonable quantities of the baccatin precursors, thus providing an abundant renewable source of taxol and related agents. In addition, millions of *Taxus* plants, representing a wide range of species and cultivars, are grown in U.S. nurseries for sale as ornamental shrubs. These, together with the mass cultivation of *Taxus* strains containing relatively high yields of taxol or its precursors by companies, such as Weyerhaeuser Company, further expand the renewable needle resource. BMS recently announced that it will be phasing out the use of bark and will no longer be collecting the bark of *T. brevifolia* growing on public, federal land; bark will be replaced by the semisynthetic route from the baccatin precursors as the major source of taxol. The baccatin precursors are currently isolated by the Italian company Indena S.P.A., from needles harvested from *Taxus* species in Europe and India.

Meanwhile, taxol and related compounds have been produced by plant tissue culture, and the scale-up production from this source is being developed by companies such as ESCAgenetics and Phyton Catalytic. The total synthesis of taxol has yet to be completed, but substantial progress has been made, starting from the abundant constituent of pine trees, pinene. While the total synthesis is unlikely to provide an economically competitive alternative to the semisynthetic approach using baccatin precursors, it might provide simpler synthetic intermediates that retain the desired anticancer activities and are more amenable to large-scale synthesis.

With positive responses being seen in the treatment of various cancers in the early clinical trials of the camptothecin derivatives, CPT-11 and topotecan (Eckhardt et al., 1992; Masuda et al., 1992; Takeuchi et al., 1992), it is likely that the demand for camptothecin will escalate. In addition, another camptothecin derivative, 9-amino camptothecin, has recently entered clinical trials. While a reasonable supply of camptothecin is currently available from Chinese and Indian sources, the NCI has acted to preclude any possible shortage of these drugs through initiation of a project for the cultivation of the source tree, *Camptotheca acuminata*, in collaboration with the USDA Forest Service. In this instance, considerable experience has been gained from USDA cultivation projects in the 1960s when camptothecin itself was a clinical candidate (Perdue et al., 1970).

Recent Developments in the NCI Natural Product Drug Discovery and Development Program

Policies for Biomass Production

The experience gained in the production of taxol highlighted the necessity for studying various methods of biomass production at an early stage of development of a new anticancer or anti-AIDS agent. To this end, the NCI has implemented a Master Agreement (MA) mechanism whereby pools of qualified organizations have been established with expertise in: the large-scale re-collection of source plant materials; the cultivation of source plants; and source plant tissue culture. The areas of cultivation and tissue culture are divided into two phases, one involving the initiation of pilot-scale studies to explore the feasibility of the techniques for production of the desired agent, and the second involving application of the methods developed in the feasibility studies to large-scale production. When a plant-derived agent is approved for preclinical development, Master Agreement Order (MAO) Requests for Proposals (RFPs) for projects in one or more of the above areas are issued to the relevant pools of MA Holders who then submit technical and cost proposals addressing the particular RFP specifications. An award is made to the MA Holder whose proposal is considered best suited to the Government's needs.

Recent Discoveries

To date, more than 35,000 plant samples have been collected by NCI contractors, and over 25,000 have been extracted to yield more than 50,000 organic solvent and aqueous extracts. Over 25,000 of these extracts have been tested in the anti-HIV screen, and about 2700 have exhibited some in vitro activity; of these, close to 2400 are aqueous

extracts, and in the majority of cases the activity has been attributed to the presence of ubiquitous chemotypes, such as polysaccharides and tannins. Such compounds are not a current NCI focus for drug development and typically are eliminated early in the discovery process.

A number of novel in vitro active anti-AIDS agents have been isolated and selected for preclinical development. The dimeric alkaloid michellamine B has been isolated from the leaves of the tropical vine *Ancistrocladus korupensis* D. Thomas & Gereau, collected in the rainforest regions of southwestern Cameroon (Manfredi et al., 1991; Thomas & Gereau, 1993). Michellamine B shows in vitro activity against both the HIV-1 and HIV-2 forms of the AIDS virus and is in advanced preclinical development. Preliminary surveys of the occurrence and abundance of *A. korupensis*, as well as cultivation experiments, have been carried out by the Missouri Botanical Garden through an extension of its current contract with the NCI. Surveys thus far indicate that its range and abundance are very limited, but fallen leaves collected from the forest floor have been shown to contain reasonable quantities of michellamine B; the collection of these leaves has obviated the large-scale harvest of fresh leaves and avoided possible endangerment of the wild species. Fallen leaf collections will provide sufficient michellamine B to complete preclinical studies, but the NCI is proceeding with feasibility studies of the cultivation of the plant through the Master Agreement process. The collections and cultivation experiments are being performed with the full participation of Cameroon authorities and scientists, as well as close collaboration with the World Wide Fund for Nature, which is coordinating conservation projects in the Korup region of Cameroon. Thus far, no other *Ancistrocladus* species have shown significant anti-HIV activity.

Calanolide A is a novel coumarin isolated from the leaves and twigs of the tree *Calophyllum lanigerum* Miq. var. *austrocariaceum* (T. C. Whitmore) P. F. Stevens, collected in the rainforest regions of Sarawak, Malaysia (Kashman et al., 1992). Calanolide A shows potent in vitro activity against HIV-1 and several resistant strains of the virus, but not against HIV-2, and is in early preclinical development. Re-collections of plant material identified as *C. lanigerum* from the same general location have shown a range of test results, varying from reasonable activity to total lack of activity. It is apparent that the production of calanolide A is dependent on various factors, possibly including the immediate growth environment of *C. lanigerum* and the time of harvest. Careful taxonomic and chemotaxonomic studies of this species are being performed by the NCI contractor, the University of Illinois at Chicago, in collaboration with the NCI and scientists from Sarawak. A survey of related species has shown that the latex of *C. teysmannii* var. *inophylloide* P. F. Stevens, collected in the same region, yields the related compound costatolide, which has significant in vitro anti-HIV activity, though being somewhat less active than calanolide A. Costatolide, or a suitable derivative, might also be considered for preclinical development. The latex contains high yields of costatolide and would be an excellent renewable source of the compound should it advance to clinical development.

A novel trimeric naphthoquinone derivative, conocurvone, has been isolated from a *Conospermum* species endemic to western Australia (Decosterd et al., 1993); this plant was collected for the NCI program by the USDA in 1981. Conocurvone exhibits potent in vitro activity against HIV-1 and is in early preclinical development. Conocurvone has been synthesized from the monomeric naphthoquinone, teretifolione B, also isolated from the plant, while simpler trimeric naphthoquinone analogs have been synthesized and shown to possess equivalent in vitro anti-HIV activity. The development of conocurvone or related compounds will be undertaken in close collaboration with Australian scientists, and surveys of the occurrence and abundance of the source plant and related *Conospermum* species are being carried out by the Western Australian Department of Conservation and Land Management (CALM).

Another potential anti-AIDS agent, prostratin, has been isolated from the stemwood of the western Samoan tree, *Homalanthus nutans* (Forster) Pax (Gustafson et al., 1992). This tree is used in western Samoa for the treatment of a variety of diseases, including yellow fever (Cox, 1990); an extract of the stemwood was provided by Paul Cox of Brigham Young University, Utah. While prostratin belongs to the phorbol class of compounds, which frequently exhibit significant tumor-promoting properties, it does not appear to be associated with tumor promotion and has been selected for early preclinical development.

Of the approximately 30,000 extracts tested so far in the in vitro human cancer cell line screen, less than 1.0% have shown some degree of selective cytotoxicity. Interesting, novel patterns of differential cytotoxicity have been observed, some of which have been associated with known classes of compounds such as cardenolides, cucurbitacins, lig-

nans, and quassinoids, while others appear to be new leads which are being investigated further.

Conclusion

Plants have played an important role in the NCI drug discovery program for over 30 years, and important plant-derived anticancer agents discovered in this program include taxol and camptothecin, which has been converted to several clinically effective analogs. The NCI also played a significant role in the development of clinically used drugs, such as vinblastine and vincristine, and the podophyllotoxin analogs, etoposide and teniposide. While considerable effort has been devoted to the production of these drugs by alternative methods, such as synthesis and tissue culture, it is significant that the wild or cultivated plants remain the major sources of either the drugs themselves or key precursors. Experience with the large-scale production of taxol has emphasized the need for the investigation of alternative sources to the wild plant at early stages of preclinical development of potential new drugs, and the NCI has introduced policies for achieving this goal. The collection and screening of plants from over 20 countries for anticancer and anti-HIV activity, and the isolation of some novel active agents, presents the prospect for the development of pharmaceutical crops in some of these countries. It must be emphasized, however, that drug discovery and development is a lengthy process requiring 10 to 20 years of intensive research, and very few new agents eventually succeed in reaching the market as effective drugs.

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EDGAR ANDERSON, BOTANIST AND CURATOR OF USEFUL PLANTS[1]

Charles B. Heiser[2]

Abstract

Edgar Anderson (1897–1969), who spent most of his life at the Missouri Botanical Garden, serving as its director from 1954 to 1957, made many contributions to economic botany and ethnobotany. Using his book *Plants, Man and Life* as a starting point, I shall discuss several of these contributions and bring in a number of personal recollections.

It was 23 years ago when I (Heiser, 1972) last presented the banquet address at this symposium. My talk was entitled "Student Days with Edgar Anderson or How I Came to Study Sunflowers." When I was asked to give the present talk, P. Mick Richardson suggested that I might want to include something about Dr. Anderson, as well as useful plants, and the more I thought about it, the prospect of devoting most of the talk to him appealed to me. I came close to using the same title as before only with the substitution of "cultivated plants" for "sunflowers," for this will be a highly personal account. In fact, I thought of calling it "Edgar and me."

Let me begin by giving a little of Anderson's background. He was born in Forestville, New York, in 1897 and moved to East Lansing, Michigan, when he was three. He attended Michigan State College (now Michigan State University) where he was a horticulture major, earning his B.S. degree in 1918. He then went to Harvard where he received the Sc.D. in 1922. He came to St. Louis in the same year and served as Geneticist at the Henry Shaw School of Botany at Washington University until 1930. The next four years he spent at the Arnold Arboretum where his title was Arborist. From there he returned to the Missouri Botanical Garden in 1935, becoming Director in 1955. After giving up the directorship in 1957, his title was Curator of Useful Plants. Upon his retirement in 1967, he continued at the Garden as Botanist, a title, like that of the previous one, of his own choosing. He died in 1969. For those who want to know more about his life, I recommend Finan's (1972) and Stebbins's (1978) accounts.

In the *American Men of Science* in 1955, he listed his research interests as: the species problem; hybridization in evolution; classification, morphology, history, and breeding behavior of *Zea mays*; and genetics of self sterility. His interests, though, were hardly covered by this list. Botanists are usually classified as belonging to one field, and if he had been asked to give his he would have given genetics, the field in which he took his Sc.D. under E. M. East. It is impossible to limit him in this way, however, for he was also a horticulturalist, an ethnobotanist (a word he seldom used), ecologist, cytologist, naturalist, and taxonomist, although he would emphatically deny that he was a taxonomist. He was remarkably versatile and I think his last title, Botanist, is the most fitting, for he was an all-around botanist, the likes of which we may never see again. To gain some idea of his breadth one need only to read the titles of his publications (Eisendrath, 1972). One may be surprised to find that the majority of his numerous papers fall into the category of popular or semi-popular writing, most of which appeared in the *Bulletin of the Missouri Botanical Garden*. Many also appeared in the *Bulletin of Popular Information of the Arnold Arboretum*, *The Herbarist*, and *Landscape*. These papers resulted from a strong commitment to the education of the interested layperson. Even the titles are often interesting. My favorite is "How to Spend a Nice Quiet Evening with a Potato" (Anderson, 1955a), followed by "Go to the Index First; Don't Use the Key Unless You Have To" (Anderson, 1955b). His collected papers are bound and may be found in the library of the Missouri Botanical Garden.

Anderson published several works that fall into the field of ethnobotany, the most important of

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which, I feel, are "Man as a Maker of New Plants and New Plant Communities" (Anderson, 1956a), "The Evolution of Domestication" (Anderson, 1959), and particularly his book, *Plants, Man and Life* (1952). No doubt many of you have read this book, and although it is now fairly old I still recommend it to those of you who have not. Many things that he says I know had an influence on me, and in rereading his book I found that there are other things that I had forgotten that also influenced me. It is probably no accident that my greatest interest in ethnobotany is the same as his—the origin and evolution of domesticated plants. I feel sure that this book was an important stimulus to the research of many others. It also perhaps tells us more about Anderson the man than any of his other writings.

Plants, Man and Life had three publishers, the first being Little, Brown and Company in 1952, and the second Andrew Mellon of London in 1954. I happened to be visiting the Garden when Anderson received a letter from the latter. He told me that the publisher wanted to arrange to have someone change the Americanisms to English, and he added that this was hardly necessary, for he had lived in England long enough to do this himself. (He had spent the academic year 1929–1930 in England.) Thus color became colour, fiber became fibre, and so on. These, as far as I can detect, are the only changes between the two printings. A paperback edition appeared in 1967, published by the University of California Press. This appears to be unchanged from the Little, Brown printing except for a new preface, an epilogue, and a few additions to the suggested readings.

For most of the remainder of my talk I would like to take up this book, chapter by chapter, touching upon various points, although not always his main ones, which will in turn lead me to related topics.

In the first two chapters of the book, "Man and His Transported Landscapes" and "The History of Weeds—A Detective Story," we learn that the "history of weeds is the history of man" and also that "the reader should be warned that there is no one to whom he can turn to for an orderly history of weeds; by a strange paradox the commonest of plants are comparatively unknown," a theme that he will return to more than once.

My interest in weeds, no doubt, stems in part from Anderson, but he is not entirely responsible. My undergraduate days at Washington University were during the war years when gas rationing was in effect; thus, although I preferred to do so, only rarely did I get into the countryside to see wild plants. So most of my field trips were to vacant lots and railroad yards around St. Louis where weeds were my subjects; as a result, my interest in weeds continues to this day.

Two of my early popular articles (Heiser, 1949, 1950) dealt with weeds, and in the former I first defined weeds as plants which grow in places in some ways disturbed by man or his domesticated animals. I would, of course, change the definition today by substituting "that" for "which" and "people" for "man."

Although I had most of Anderson's reprints in my collection, it was only much later that I became aware that I was lacking his "A Classification of Weeds and Weed-like Plants" published in *Science* in 1939. In it Anderson tells us that weeds are "plants unintentionally grown by man, in fields, gardens, pastures, lawns, etc."; ruderals are plants that spread "into man-created habitats (barnyards, roadways, dumps, etc.) though not actually cultivated"; and nomads are plants that "spread widely and rapidly even when not associated with man. The last are species for the most part of river valleys, seashores and other habitats marked by bare and shifting soils." Here belong many potential weeds and "apparently was the source of many of our weeds." I had learned from Anderson early on that weeds had a close relation to domesticated plants, that sometimes weeds gave rise to cultivated plants, and that sometimes domesticated plants gave rise to weeds.

In the third chapter, entitled "The Greater Paradox," Anderson starts by saying that "the great paradox that our commonest plants are the least known has given rise to a greater one, that this perilous situation is generally unsuspected." He goes on to say that most scientists assume that the classification of cultivated plants is "tolerably well understood." Nothing is farther from the truth, and the average taxonomist "prefers not even to think of them." He goes on to state that there are logical reasons for taxonomists' avoidance of cultivated plants—one being that they are more difficult than wild plants—and that the remainder of the chapter will be a defense of taxonomists. Maybe he really did think that but I, and I think many other taxonomists, felt that most of the chapter was more of a stinging criticism of taxonomists.

It is in this chapter that maize, the plant that was to occupy most of Anderson's research attention for the remainder of his life, is first mentioned, and he writes that he became interested in the classification of maize some ten years earlier. I have not been able to determine exactly when he decided to make it his principal research subject.

Many of East's students at Harvard worked with maize, but *Nicotiana* served for Edgar's thesis. In 1939 Mangelsdorf and Reeves listed him in their acknowledgments, stating that he "has followed our investigations for many years and has made numerous critical and stimulating suggestions." One of these was that instead of being the progenitor of maize, teosinte might be a hybrid of maize and *Tripsacum*. This became the basis for Mangelsdorf's, now largely discredited, theory for the origin of maize. In a paper in 1969, Anderson wrote that when Mangelsdorf and Reeves demonstrated that corn and *Tripsacum* could be crossed he started to study them "as a taxonomist would study grasses that interested him."

Beginning in 1940, we find maize or *Zea* listed in Anderson's publications and in that year he published "Sweet Corn with a College Education" in the *Bulletin*; this was not concerned with his own research but with new hybrid sweet corns for the home garden. From that year on he published regularly on maize. His first paper, with Hugh Cutler, on the classification of the races of *Zea mays* appeared in the *Annals* in 1942. I recall that in 1945, in my cytology course with him, we spent the entire semester studying meiosis in *Zea mays*. Earlier that year he and William Brown had spent some time at Cold Spring Harbor with Barbara McClintock, and he wrote me that we "are now turning out corn smears that would make any cytologist turn green with envy."

In a paper on maize in 1968, Anderson begins, "During the years when research on corn and its relatives was my main interest, I was often introduced to audiences as 'An authority on corn,' to which I usually responded that I was NOT an authority on corn, but an authority on what was not known about it. I meant every word." Although he was emphatic, I must disagree, for he *was* an authority on corn.

In 1969, in his last paper on maize, he tells us how his association began with the Pioneer Hi-Bred Corn Company, which supported his work and that of his students for many years with no strings attached. He tells us that Henry Wallace, the founder of the company, had told his colleagues that "this company was founded on a theory that everyone thought was crazy. We should be receptive to crazy ideas about corn breeding." The money they spent on Anderson and his students was done without any formal agreement. It was money well spent, for not only did he provide their breeders with many ideas but three of his students, William Brown, Donald Duvick, and Harold Kidd, became associated with the Pioneer Hi-Bred Corn Company as breeders; Brown later became president of the company. I am sure that Edgar took great satisfaction in knowing that he had contributed to the improvement of one of the world's most important plants, for I know the thought of improving a plant useful to people has great appeal to me.

The next chapter on "The Clues from the Root Tips" deals with the use of chromosomes in taxonomy and the study of the origin of domesticated plants. Anderson was one of the pioneers in the use of chromosomes in taxonomy and one of the founders of the field of biosystematics. This is followed by a chapter on "How to Measure an Avocado" in which he begins by saying that "it is the simplest questions which are the hardest to answer" and then goes on to introduce some of the simple pictorial devices that he had invented to study variation.

In Chapter 7, "Budgets and Scholarships," Anderson begins, "By this time it is clear to me, and I think it should be evident to the reader, that this book is getting a little out of hand," and my talk, I fear, is also. In this chapter, he tells us that biology is only one part of studying the origin of cultivated plants: "It is quite as much a problem in history, in archaeology, in anthropology, in nutrition, in sociology. Problems which fall straight across department lines run into administrative red tape." Later he continues that "if the study of cultivated plants is to advance as it should one must find ways to fuse interdepartment interests over a broad front. Knowledge is all one piece but universities (by tradition and for budgetary reasons) are divided into departments." Still further along he states that "the work will have to be done by men or groups of men whose understanding embraces these several disciplines." Perhaps unknown to him, interdisciplinary teams had already started working on archaeological investigations, the earliest I know of being that of Robert Braidwood (1972) in the Near East. Since that time teams have become rather common, with fruitful results for our knowledge of the origin of agriculture and domesticated plants.

In the same chapter, after a discussion of primitive music, Anderson writes that "From the evidence already at hand it is evident that magic had as much to do with primitive domestication as with primitive music. Body paints, of magical or religious significance, charms, rattles, magic cure-alls, certainly had as much to do with the origins of food plants as the utilitarian need of food which the armchair experts have stressed in their pronouncements." For some time I have felt that primitive religion was somehow involved in the origin of

agriculture (Heiser, 1973, 1985). Had someone asked me a year ago I would have said that the reading of Sir James Frazer's *The Golden Bough* (Gaster, 1959) had turned my attention in that direction, but now I am not so sure; it may have been Edgar.

Chapter eight is entitled "Uneconomic Botany." Anderson and other students in the course in Economic Botany at Harvard referred to it as Uneconomic Botany, for their professor, Oakes Ames, one of the people to whom *Plants, Man and Life* is dedicated, spent little time on the most basic food plants, preferring to discuss such subjects as arrow poisons and amber. Anderson goes on to tell us that some of the topics he once considered as uneconomic he now considers of promising economic importance and that the course he once considered so useless, he now realizes was his most practical.

In this chapter he also states that it is difficult "to define the exact limits between weeds, cultivated plants and truly wild species," a problem that many of us still face. He also introduces us to "encouraged" weeds, a term that I have found most useful in describing some of the plants I work with.

The chapter that today still receives considerable attention is his "Dump Heaps and the Origin of Agriculture," most recently by Bruce Smith in his *Rivers of Change* (1992). He uses Anderson's "open habitats" and "floodplain origin of weeds" as cornerstones in his own "floodplain weed theory of plant domestication" for eastern North America. When I first read Anderson's chapter I did not fully appreciate it, for at the time I was not particularly interested in the origin of agriculture. His ideas on the subject were new to me at the time, for I had never heard him discuss them in classes or in conversations with me.

He starts the chapter by discussing his sojourns in Mexico and Guatemala where the natives' gardens appeared to be nothing "but dump heaps and a few trees in yards behind their houses," and only later did he realize that "many of these dump heaps were carefully managed gardens and orchards. . . ." He goes on to say that "these hit-or-miss gardens are of particular significance because they fit in perfectly with a theory of Carl Sauer's that agriculture must have originated among sedentary fisherfolk." Sauer is another person to whom the book is dedicated.

I am sure that many of you are well acquainted with Anderson's dump-heap theory, but let me quote what I think are the most pertinent passages.

> To me this theory is worth careful consideration because such people would have also created refuse heaps and I am even more intrigued than Sauer by the notion that such refuse heaps may have played a key role in the origin of cultivated plants. These ancient dump heaps are of extraordinary interest in connection with the origin of cultivated plants, because they are open habitats.
>
> Here is a strange new kind of habitat. Many of the plants in the native flora do not fit into it, some aliens will, and some hybrids. Plants which can grow in such places will have less interference from other plants. Kitchen middens would be likely places in which fruit pits, seed heads, and the like, brought to the village from some distance, might germinate and survive.
>
> If we now reconsider the kitchen middens of our sedentary fisherfolk, it seems that they would be a natural place where some of the aggressive plants from the riverbanks might find a home, where seeds and fruits brought back from up the hill or down the river might sometimes sprout and to which even more rarely would be brought seeds from across the lake or from another island. Species which had never intermingled might do so there, and the open habitat of the dump heap would be a more likely niche in which strange new mongrels could survive than any which had been there before man came along. Century after century these dump heaps should have bred a strange new weed flora and when man first took to growing plants, these dump-heap mongrels would be among the most likely candidates.

Both Sauer's and Anderson's hypotheses came along early in attempts to explain the origin of agriculture. Since that time there have been many endeavors, including several books on the subject, but there is still no agreement as to the cause, or causes, for the origin of agriculture, although we now have, thanks to the archaeologists, good knowledge as to where agriculture first made its appearances.

By the time I could have discussed the subject with Anderson intelligently, it was too late. I did not become aware that he had written more on the topic until recently, when I read in the epilogue of the paperback edition of the book that he had written several essays in the journal *Landscape* that are related to matters discussed in the book, including one that "takes up in more detail the 'dumpheap theory' of the origin of cultivated plants." He states that references to these are given in the suggested readings. Only they aren't. I traced it down to a paper entitled "Reflections on Certain Honduran Gardens" (Anderson, 1954). If you are interested in the subject it would pay you to read it, but all that I am going to quote here are his opening remarks, for many of them are still pertinent today.

> For somewhat over a decade I have been dipping

> here and there into the technical literature about the origin of agriculture. Some of the pronouncements made me squirm a little, intellectually. They seem bookish and doctrinaire; there is no flavor of the hoe about them. None of them have the air of having been written by a man who had just come back into the house after thinning the radishes or dusting the young cabbages. They distinguish field culture from garden culture; they use precise terms appropriate for European conditions in the last few hundred years. As for me, I am not so sure.

Although the title "dumpheap origin" belongs to Anderson it so happens that a somewhat similar hypothesis for the origin of domesticated plants was proposed by the German geographer Th. H. Engelbrecht, in 1917. I am not sure where I first came across the reference but it probably was in Darlington (1956), which I have in my own library. The paper was in German so I had a copy made, which I took home to my wife for translation. A published translation has since appeared (Zeven, 1973). Engelbrecht writes that primitive people gathered roots and tubers that were brought to their homes. Their refuse built up a habitat for a weedy flora in the vicinity of their dwellings without aid of the people. Many of the plants were useful and became the oldest stock for our cultivated plants. When such plants were treated with care and occasionally weeded, and a prearranged planting replaced their haphazard dispersal, then cultivation is imperceptibly under way.

It is easy to see how vegetatively propagated domesticated plants could have evolved in a dumpheap habitat, for a superior mutant form would have immediately bred true. For seed propagated plants it is more difficult, for not until seeds were saved and deliberately planted could unconscious or conscious selection operate to give rise to stabilized superior new forms (Heiser, 1990). Thus when and why seeds were planted remain the big questions.

Chapter 10 on 100 of the world's most important crop plants is the part of the book that is most outdated. The "scandalous," as he called it, situation was how little was known about the origin of so many of them. By 1976, when Simmonds's book appeared, the situation was considerably better, no doubt owing partly to Anderson's challenge. Since that time we have learned still more about the origin of several domesticated plants. For many, however, we still know very little, and our knowledge of others may have to be revised as they are studied by molecular techniques.

The penultimate chapter is about my favorite plant, the sunflower, and much of it concerns my own research. I believe it was Herb Wagner who once said that Anderson's chapter did more for me than all of my own writings. It was, of course, a comment of Anderson's (Heiser, 1972) that led me to study the plant. I was fortunate that he did not keep it for himself, for upon rereading this chapter I now feel that it was also his favorite plant. Listen to this:

> Ever since I first saw acres of wild sunflowers blossoming in gold and green confusion all down the back slopes of Dago Hill in Saint Louis, I have been intoxicated with sunflowers. They happen to be of key significance in the story of plants and man; if they were not I should be tempted to tell you about them anyway. I wish it were possible fully to communicate my enthusiasm for these lusty brilliant flowers, these coarse and resinous weeds which have been so closely tied up with man during his varied history on this continent. Crop plant, garden flower, roadside weed, growing unasked but seldom unwanted on vacant lots and rights of way. I wish one could in a few sentences paint the full pageant of them in any one year. The colonies of them in railroad yards in our larger eastern cities, the thousands upon thousands of acres in the Great Plains, long golden rows of them bordering roadsides and drainage ditches throughout the West, regular lines of them planted as windbreaks in some of the irrigated valleys, little bunches of them in neat Hopi gardens below the mesa tops, acre after acre grown for oil in western Canada.

Except in a very few places this chapter stands up very well today, nearly a half century after it was written. One big mistake is in the title, "Sunflowers—the One Native American Crop." What Anderson meant, of course, was the one native North American crop. However, it appeared unchanged in all of the printings and I was never aware of the error until someone else pointed it out to me.

In the early fifties, inspired by Anderson's frequent statements that we needed to know more about the process of domestication, I set up a little experiment. Using seeds of weed sunflowers from St. Louis I decided to try to recreate the domesticated sunflower. By crossing the most promising plants, after four generations I had unbranched sunflowers with heads and achenes considerably larger than those of the weed sunflowers. I then gave up the experiment, for I realized that although I had never seen a hybrid of a weed and domesticated sunflower in the St. Louis area, there was no way in which I could prove that my original seed had not been contaminated with the cultivated sunflower in the past.

In the copy of *Plants, Man and Life* that Edgar gave to me he had written, "Some day this will

grow into a whole book on The Sunflower, and you, I hope, will write the book." I am happy to say that I have written such a book (Heiser, 1976), but I regret that it did not appear until after his death. I am sure that he could have brought up many questions that I failed to answer.

In fact, in his own final chapter Anderson tells us that his book is largely about what we do not know, which is one of the reasons one finds his book so stimulating. The chapter is entitled "Adventures in Chaos," and the meaning of the title, he explains, is that "the study of cultivated plants is an adventure in apparent chaos." A little later he goes on to say that the only "branch of biology that is equipped to deal with such work is taxonomy." Then again after stating that he is not a taxonomist, he writes that he was fortunate very early to acquire a respect for the taxonomic method. "It was probably no accident," he states, "that Dr. Alfred C. Kinsey and I, who were graduate students together, should both be using the taxonomic method where it has not recently been generally used, he in the study of sex and I in the study of cultivated plants."

After I came to Indiana University, I became acquainted with Kinsey, and he amplified Anderson's statement, telling me that he had been successful in the study of human sexual behavior where others had failed by using the taxonomic method that he had learned from M. L. Fernald at Harvard. He also once told me that gall wasps were better at recognizing the species of oaks than were plant taxonomists.

There is much more that I would like to quote from Anderson's final chapter but I shall let you discover these things yourselves so that I may have time to say a little more about Anderson, the teacher and the person.

I was reading his description of his teaching method (Anderson, 1956b) on the same day I was reading a new Sherlock Holmes work. Anderson related how he would take students into the field and tell them that "their assignment for the next hour is to find out what were the main steps in vegetational change on this site in the last century. . . . Don't neglect any kind of evidence, just because it seems trivial." That reminded me of Holmes telling Watson not to overlook any clue, no matter how insignificant.

In another place Watson tells us that there was always a touch of the dramatic in Holmes. In Anderson it often was more than a touch, which reminds me of the story Stebbins (1972) tells in Anderson's words.

> When I was in Berkeley during the war, Ledyard and I used to have lunch together in the warm sunny alcove outside of his laboratory on the top floor of Hilgard Hall. We had many good talks together, but I'll never forget his reaction to something that I did a little while after I arrived in California. You see, almost all of the men were gone, and I had heard that the sororities were having trouble in finding help. So I went to one of them, offered my services, and they hired me as a waiter. When I told Ledyard this, he looked at me, all of the blood of his strict New England ancestors curdled in his veins, and he blurted out: "Edgar, you'd do *anything* to be bizarre, wouldn't you?"

One thing that was largely lacking in Holmes, however—a sense of humor—was well developed in Anderson.

From a postcard to me—"My field botany course begins today and we shall be studying sunflowers. . . . You know, those things with the big yellow flowers."

At times I thought his humor could be a little biting. I gave him a draft of one of my first papers to read concerning the Hopi sunflower, only two pages long but half of the second page was devoted to acknowledgments. When he returned it I found that he had added, "and to God for the sunshine, and to the Devil for the rain." I shortened the acknowledgments.

Once after Edgar had been to a large university to give a seminar, he told us, "You know when you go into the greenhouses at a university you can tell to whom a particular room belongs. If the plants look terrible but are all correctly identified, it belongs to the taxonomist; if the plants are beautiful but misidentified, it belongs to the horticulturalist; and if the plants look bad and are mostly incorrectly determined, it is the house of the plant physiologist." I have no idea as to why I have remembered this story for half a century.

It is time for me to stop. I have not told you everything that I know about him, but I want to save some things for the next time I am invited to speak on Anderson at this symposium—say about 23 years from now.

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BIOLOGIA FLORAL Y POLINIZACION DE ALGUNAS MONOCOTILEDONEAS DE UN BOSQUE NUBLADO VENEZOLANO[1]

Alberto Seres[2] *y Nelson Ramírez*[2,3]

Resumen

La biología floral y los mecanismos de polinización fueron estudiados en 33 especies de monocotiledóneas, principalmente herbáceas, del bosque nublado del Parque Nacional Henri Pittier, Venezuela. La distribución de frecuencia de los modos de polinización muestra una marcada tendencia a la polinización por coleópteros (35.1%), aves (24.3%), y abejas (16.2%); dipteros (10.8%), viento (8.1%), y murciélagos (5.4%) fueron menos frecuentes. De 71 especies de agentes visitantes registrados en 33 especies de plantas, el 67.6% fueron polinizadores efectivos y 32.4% fueron sólo visitantes. Los coleópteros y los dípteros representaron los grupos más diversos. El 46.9% de las especies fueron monoicas-dicógamas y el 53.1% hermafroditas-adicógamas; la protoginia (37.5%) fue más frecuente que la protandria (9.4%). Entre las especies adicógamas, el 15.6% eran hercógamas. La flor es la unidad de polinización en la mayoría de las especies hermafroditas y monoicas. Las plantas polinizadas por aves, abejas, y murciélagos producen néctar como recompensa, aunque el polen puede ser la recompensa en especies melitófilas y quiropterófilas. En las plantas cantarófilas, los coleópteros comen partes florales. La longevidad de las flores polinizadas por vertebrados fue de 10 a 24 horas, mientras que en las especies melitófilas fue más variable. La longevidad de la unidad de polinización en especies polinizadas por coleópteros fue mayor, principalmente asociada a la extensa longevidad de las flores femeninas. Una situación similar ocurrió en especies anemófilas. Las diferentes pautas encontradas en la hora de antesis, longevidad floral, especies polinizadoras, junto a la distribución vertical y horizontal de las especies fueron considerados algunos de los mecanismos que promueven la especificidad de polinización y el flujo de polen intraespecífico.

Abstract

Floral biology and pollination mechanisms of 33 herbaceous monocot species were studied in the tropical cloud forest of Henri Pittier National Park, Venezuela. The frequency distribution of pollination systems was as follows: cantharophily (35.1%), ornithophily (24.3%), melittophily (16.2%), myiophily (10.8%), anemophily (8.1%), and chiropterophily (5.4%). Of 71 visiting species recorded on 33 plant species, 67.6% were pollinators and 32.4% were only visitors; coleopterans and dipterans were the most diverse groups. Studies of floral biology showed that 46.9% of the species were monoecious-dichogamous and 53.1% were hermaphroditic-adichogamous. Among adichogamous species, 15.6% were herkogamous. The pollination units were the flowers or inflorescences in hermaphroditic and monoecious species. Bat-, bird-, and bee-pollinated species produced nectar, although pollen was also found as a reward in bee- and bat-pollinated species; beetle pollination was associated with floral parts as the reward. Floral longevity of vertebrate-pollinated species ranged from 10 to 24 hours; floral longevity for bee-pollinated species was similar or longer than for vertebrate-pollinated species. For cantharophilous and wind-pollinated species, pollination

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unit longevity was increased by the longevity of the female flowers. These different patterns in time of anthesis, floral longevity, and pollinator species, together with vertical and horizontal species distribution, are mechanisms that promote pollination specificity and intraspecific pollen transportation in the understory cloud forest.

En los estratos bajos de bosques tropicales se han examinado las estrategias florales y modos de polinización en especies aisladas (Wasshausen & Arroyo, 1976; Beach, 1982), grupos taxonómicos de plantas (Soderstrom & Calderon, 1971; Stiles, 1975; Bullock, 1981; Sobrevila et al., 1983; Renner, 1984; MacDade, 1985) y gremios particulares de polinizadores (Stiles, 1977; Feinsinger, 1978; Feinsinger et al., 1986). Sin embargo, estudios comparativos que muestren las diversas estrategias que promueven el flujo de polen intraespecífico en especies de plantas con diferentes modos de polinización, biología floral y sexualidad son prácticamente inexistentes en especies herbáceas de bosques tropicales.

La dinámica de polinización en el contexto de los mecanismos que promueven el flujo de polen intraespecífico y la especificidad de polinización es poco conocida en bosques tropicales. Sin embargo, el desplazamiento en los períodos de floración, el sistema de entrecruzamiento y el comportamiento de los polinizadores evidencian que el flujo de polen pueda ser extenso en especies de árboles tropicales (Ashton, 1969; Bawa & Opler, 1975; Bawa, 1980; Frankie et al., 1976; Sobrevila & Arroyo, 1982). En contraste, algunas monocotiledóneas herbáceas estudiadas en el bosque nublado Henri Pittier presentan períodos de floración y fructificación extensos y superpuestos (Seres & Ramírez, 1993) y además, son principalmente autocompatibles (Ramírez & Seres, 1994). Estas pautas sugieren que el desplazamiento temporal fenológico y el sistema genético de reproducción deben estar asociados a estrategias florales y de polinización que promuevan el flujo de polen intraespecífico. El estudio de la biología floral y polinización de monocotiledóneas con distintos modos de polinización podría evidenciar los mecanismos a través de los cuales se promueve el flujo de polen intraespecífico en los diferentes gremios de polinizadores.

La sexualidad, dicogamia, tipo de recompensa, hora de antesis y longevidad floral son caracteres considerados en este trabajo como importantes en la biología de polinización. Además, el transporte diferencial de las cargas de polen sobre el cuerpo del agente visitante puede contribuir a reducir la interferencia entre especies (Feinsinger et al., 1986; Ramírez, 1989). La distribución espacial de las especies puede ser una característica que contribuye a reducir la interferencia reproductiva entre las especies herbáceas del bosque nublado.

Area de Estudio

Este trabajo fue realizado en el Parque Nacional Henri Pittier, Rancho Grande, en los bosques adyacentes a la Estación Biológica de Rancho Grande (10°21'N, 67°41'O), aproximadamente a 12 km de Maracay, Edo. Aragua, Venezuela. El parque se encuentra en la zona central de la Serranía del Norte de la Cordillera de la Costa, abarcando extensas zonas de ambas vertientes (norte y sur). El área de estudio abarcó altitudes entre 1000 y 1400 m, y el estudio se realizó en las siguientes zonas locales: (1) camino de la toma de agua; (2) vía Pico Cumbre Rancho Grande hasta 1400 m; (3) Paso Portachuelo; y (4) vía Pico Periquito hasta 1350 m. Las tres localidades incluyen principalmente bosques primarios.

El área de Rancho Grande se encuentra en la zona de vida de bosque muy húmedo premontano (bosque nublado), con precipitación que varia entre 2000 y 4000 mm anuales, y una temperatura media entre 18° y 24°C (Ewel et al., 1976). En el bosque nublado de Rancho Grande se observa una estacionalidad con un período seco corto a principios de año (enero–marzo), aumentando la precipitación hasta su máximo en agosto y septiembre, y disminuyendo hacia finales de año. Durante los meses secos la formación y presencia de neblinas y "lluvias ocultas" mantienen una alta humedad ambiental con un aspecto de bosque siempre verde.

Estudios ecológicos recientes en el Parque Nacional Henri Pittier (Huber, 1986) han demostrado la presencia de distintos tipos de bosques nublados: bosque nublado de transición, bosque nublado propiamente dicho, y bosque nublado superior. Este trabajo abarca los dos primeros tipos de bosque nublado. El bosque nublado de transición está constituido por tres estratos arbóreos y uno herbáceo-arbustivo entre los 0.5 y 3.0 m que forman una cobertura irregular y densa, en la que se encuentran especies de palmas y varios arbustos. Sin embargo, las hierbas altas son las que caracterizan este estrato y falta casi por completo el estrato herbáceo a nivel del suelo (Huber, 1986). El bosque nublado propiamente dicho está formado por dos estratos arbóreos. Los estratos arbustivos y her-

báceos son generalmente más densos que los del bosque nublado de transición.

Metodos

La selección de las especies estudiadas fue de acuerdo a su frecuencia y conspicuidad. Cuatro transectas de 200 m de longitud fueron colocadas en cada una de las localidades del bosque. De las especies herbáceas y sub-herbáceas más frecuentes a lo largo de cada transecta se seleccionaron las monocotiledóneas más conspicuas, esto basado en la altura y cobertura.

Las muestras botánicas de las especies estudiadas están depositadas en El Herbario Nacional de Venezuela (VEN). Las muestras de los insectos visitantes y polinizadores están depositadas en los museos a los cuales están asociados los especialistas que realizaron las identificaciones (ver sección de agradecimientos). Los especímenes de aves y murciélagos están depositados en El Museo del Ministerio del Ambiente y los Recursos Renovables.

La información sobre la distribución vertical de las especies, así como la distribución en subambientes de las 33 especies estudiadas del bosque nublado de Rancho Grande fue recopilada por Seres & Ramírez (1990). La distribución vertical de las especies muestra que las monocotiledóneas estudiadas están principalmente distribuidas en el estrato herbáceo del bosque: 69.7% (N = 23) son hierbas entre 0.3 y 4.0 m de altura; 9.1% (N = 3) son trepadoras herbáceas que despliegan sus estructuras reproductivas entre 1.0 y 10.0 m de altura (Tabla 1). Tres especies de epífitas (9.1%) están distribuidas entre 0.5 y 40.0 m de alto. Además, se estudiaron cuatro especies de palmas (12.1%), tres de las cuales tienen alturas menores de 3.0 m, y *Socratea* sp. que es una palma del dosel (± 20 m alto) (Tabla 1).

SEXUALIDAD Y DICOGAMIA

La sexualidad de las especies vegetales fue determinada en el campo y posteriormente confirmada en el laboratorio. Las especies fueron clasificadas en hermafroditas, monoicas, y dioicas. La variación temporal en la maduración sexual se determinó directamente en el campo; observaciones a intervalos de una hora durante la vida de las flores o inflorescencias (dependiendo del tipo de unidad de polinización) permitieron reconocer tres categorías: adicógamas (= homógamas), protandras (la fase masculina ocurre primero que la femenina), y protóginas (la fase femenina ocurre primero que la masculina). La condición dicógama fue establecida independientemente si había solapamiento de las fases sexuales durante un breve período de la vida de las flores o inflorescencias (dicogamia incompleta; Lloyd & Webb, 1986). Además, se determinó la presencia de hercogamia (separación espacial en la presentación del polen y el estigma) cuando el estigma estaba ubicado al menos 2 mm por encima de los estambres, antes o durante la polinización.

UNIDAD DE POLINIZACION

El tipo de unidad de polinización (flor o inflorescencia) se determinó en base a la morfología y comportamiento de dichas estructuras durante el período completo de actividad floral, relacionado a la actividad de los polinizadores.

ANTESIS Y LONGEVIDAD

Se fijó como hora de antesis, para las especies hermafroditas en cuyas flores se pueden observar los movimientos de sus partes (corola, estambres, y estilo), como la hora de máxima apertura de dichas partes florales. En especies monoicas, como las Araceae y Cyclanthaceae, se consideró la hora de antesis de las flores femeninas, cuyo perianto es reducido o ausente, como el momento en que la inflorescencia comienza a producir el perfume que atrae a los polinizadores, ya que la atracción de los polinizadores supone capacidad de recibir el polen y probablemente la receptividad estigmática en las flores femeninas. Para las flores masculinas, la antesis se consideró como el momento de la aparición del polen (dehiscencia de las anteras). La longevidad máxima de las flores e inflorescencias, ésta última cuando es la unidad de polinización, fue considerada desde el momento de la antesis hasta el cierre de las flores, la caída de éstas o cualquier señal de marchitez.

Dependiendo de las horas del día en las que se observó la actividad floral, se consideraron tres grupos de plantas: en las diurnas, la actividad está restringida a la luz solar; en las nocturnas, la actividad polinizadora ocurre al anochecer o en la oscuridad; y las diurnas-nocturnas mantienen las flores o inflorescencias activas tanto de día como de noche, y generalmente son flores con longevidad mayor a 24 horas.

ATRACCION Y RECOMPENSA

La recompensa ofrecida por las flores o inflorescencias, tales como néctar, polen, o partes florales, se determinó directamente en el campo por medio de la observación de las flores, del comportamiento de los polinizadores, y la revisión de es-

tructuras florales para precisar exactamente la recompensa ofrecida. Además, la presencia de fragancia fue detectada cuando presente.

POLINIZADORES Y VISITANTES

Las especies polinizadoras y modo de polinización fueron estimados por observación directa de la actividad de los agentes visitantes, posición y abundancia de las cargas de polen sobre el cuerpo del polinizador, todo esto relacionado con todos aquellos parámetros mencionados anteriormente. La relación entre tamaño flor-polinizador, posición de las cargas de polen, y el comportamiento durante la visita fueron los principales criterios para diferenciar visitantes de polinizadores. En todos los casos, los agentes se atraparon con métodos adecuados para su posterior identificación. En especies en las cuales se sospechaba la polinización por el viento, se hizo una prueba especial que consiste en cubrir las flores femeninas con una malla fina que permite pasar el polen transportado por el viento, sin permitir la visita de animales antófilos. Posteriormente se comparó la producción de frutos con controles naturales.

RESULTADOS

La biología floral de 33 especies de monocotiledóneas del bosque nublado de Rancho Grande está detallada en la Tabla 1. La lista de especies de plantas y especies polinizadoras junto a las características del transporte de polen están en el Anexo I.

ATRACCION Y RECOMPENSA

En Cyclanthaceae y Araceae la fragancia floral era diferente a la sensibilidad humana. En la muestra examinada existe una correlación entre el tipo de recompensa y el grupo de agentes polinizadores (Tabla 1). En las especies cantarófilas, los escarabajos consumían partes florales. Además, en las Araceae y Cyclanthaceae los coleópteros frecuentemente se apareaban en las inflorescencias. En las Cyclanthaceae los coleópteros ovopositaban los ovarios de las flores femeninas. El desarrollo de las larvas de los coleópteros era completado a expensas de partes del fruto y semillas. En el caso de las palmas cantarófilas, los escarabajos consumían polen y néctar.

Las monocotiledóneas quiropterófilas, melitófilas, y ornitófilas producían néctar como recompensa, aunque en el caso de las especies polinizadas por abejas, el polen era una fuente alimenticia adicional. Las especies quiropterófilas y ornitófilas producían abundante néctar, mientras que en las especies melitófilas las cantidad de néctar producido era mucho menor.

UNIDAD DE POLINIZACION

La flor representó la unidad de polinización en la mayoría de las especies monoicas y hermafroditas. Sin embargo, la inflorescencia era la unidad de polinización en todas las especies de Araceae y Cyclanthaceae (Tabla 1).

POLINIZACION DIURNA, NOCTURNA, Y DIURNA-NOCTURNA, ANTESIS Y LONGEVIDAD

La proporción de plantas cuya actividad de polinización estaba restringida a las horas de la noche (N = 2; 6.3%) fue comparativamente menor al de las plantas con polinización estrictamente diurna (N = 13; 40.6%). El grupo de plantas con una actividad de polinización diurna y nocturna fue la más numerosa en la muestra examinada (N = 17; 53.1%).

La antesis de las especies melitófilas ocurrió entre las 5:00 y 12:00 horas, con una longevidad de 7 a 72 horas. En las especies quiropterófilas, la antesis ocurrió entre las 17:30 y 19:00 horas con una longevidad de 10 a 12 horas (Tabla 1). La mayoría de las especies ornitófilas abrían sus flores entre las 3:00 y las 10:00 horas, con un tiempo de longevidad entre 8 y 19 horas. Sin embargo, la antesis puede ocurrir en horas de la tarde (en *Renealmia nicolaioides* Loes.), en cuyo caso la longevidad era menor (máximo de ocho horas).

Las especies cantarófilas protóginas se caracterizaron por presentar un desfasaje de aproximadamente 24 horas entre la antesis de las flores femeninas y las masculinas. La antesis de las flores masculinas y femeninas ocurrió en horas de la mañana en Cyclathaceae y Palmae, y la antesis de las flores masculinas y femeninas en horas de la tarde en Araceae (Tabla 1). Sin embargo, en *Cyclanthus bipartitus* Poit. (Cyclanthaceae), la antesis ocurrió como en las Araceae. La hora de antesis estaba relacionada con los grupos taxonómicos de polinizadores: antesis en la mañana con curculionidos y antesis en la tarde con scarabaeidos. La longevidad de las flores femeninas fue mayor que la de las flores masculinas en todas las especies cantarófilas (Tabla 1).

Las especies miófilas tienen antesis en horas de la mañana o en la tarde. Las flores masculinas abrían entre las 5:00 y 8:00 horas y las femeninas entre las 6:00 y las 18:00 horas; la longevidad de

TABLA 1. Sexualidad, variación temporal en la maduración sexual, biología floral y altura de las estructuras reproductivas de 33 especies vegetales.

Familia Especie	Sexualidad[1]	Expresión sexual[2]	Unidad de polinización[3]	Hora de antesis	Período de vida floral[4]	Longevidad máxima (h) Flor	Longevidad máxima (h) Unidad	Recompensa	Altura de las estructuras reproductivas (m)	Espécimen de referencia[5]
Araceae										
Dieffenbachia sp.	M	protog.	I	(f) 16:00–18:00	(f) n–d	(f) 18–20	36	estaminodios	1.0–2.0	AS-244
				(m) 15:00–17:00	(m) n–d	(m) 4–5				
Philodendron macroglossum	M	protog.	I	(f) 18:00–19:30	(f) n–d	(f) 18	36	flores	1.0–6.0	AS-214
				(m) 19:30–20:30	(m) n–d	(m) 3		estériles		
Xanthosoma undipes	M	protog.	I	(f) 17:00–18:00	(f) n–d	(f) 18–20	24	flores	1.0–3.0	JS-95828
				(m) 17:00–18:00	(m) n–d	(m) 4–5		estériles		
Bromeliaceae										
Aechmea lasseri	H	homog.	F	05:00–05:30	d	17–19	—	néctar	1.0–10.0	JS-89806
Pitcairnia altensteinii	H	homog.	F	05:00–06:00	d	17–19	—	néctar	0.5–1.0	AS-150
Vriesea platynema	H	homog.	F	17:30–18:30	n	12	—	néctar	3.0–40.0	JS-87810
Vriesea splendens	H	homog.	F	18:00–19:00	n	10	—	néctar	0.5–8.0	AS-256
Commelinaceae										
Tradescantia zanonia	H	homog.	F	07:00–08:00	d	8–10	—	polen	0.5–1.0	AS-79
Cyclanthaceae										
Asplundia caput-medusae	M	protog.	I	(f) 05:00–06:00	(f) d–n	(f) 18–20	30	perianto	1.0–2.0	JS-114237
				(m) 03:00–05:00	(m) d–n	(m) 4–6		anteras, polen		
Asplundia fendleri	M	protog.	I	(f) 04:00–05:00	(f) d–n	(f) 18–20	30	perianto	1.0–2.0	AS-218
				(m) 02:00–04:00	(m) d–n	(m) 4–6		anteras, polen		
Asplundia moritziana	M	protog.	I	(f) 04:00–06:00	(f) d–n	(f) 18–20	30	perianto	2.0–10.0	OH-336
				(m) 04:00–06:00	(m) d–n	(m) 4–6		anteras, polen		
Asplundia sp.	M	protog.	I	(f) 04:00–06:00	(f) d–n	(f) 18–20	30	perianto	0.5–1.5	AS-179
				(m) 04:00–06:00	(m) d–n	(m) 4–6		anteras, polen		
Cyclanthus bipartitus	M	protog.	I	(f) 15:00–18:00	(f) d–n	(f) 19–21	28	polen	0.4–1.0	AS-344
				(m) 16:00–18:30	(m) d–n	(m) 2–3		bractea		
Evodianthus funifer	M	protog.	I	(f) 05:00–07:00	(f) d–n	(f) 18–20	30	perianto	1.5–8.0	AS-220
				(m) 05:00–07:00	(m) d–n	(m) 4–6		anteras, polen		
Cyperaceae										
Scleria latifolia	M	protog.	F	(f) 06:00–15:00	(f) d–n	(f) 72	—	ninguna	0.5–1.0	AS-184
				(m) 07:00–09:00	(m) d	(m) 8–10				

TABLA 1. Continuada.

Familia Especie	Sexual-idad[1]	Expresión sexual[2]	Unidad de polinización[3]	Hora de antesis	Período de vida floral[4]	Longevidad máxima (h): Flor	Longevidad máxima (h): Unidad	Recompensa	Altura de las estructuras reproductivas (m)	Espécimen de referencia[5]
Gramineae										
Olyra latifolia	M	protog.	F	(f) 08:00–16:00	(f) d–n	(f) 72	—	ninguna	0.5–2.0	AS-156
				(m) 08:00–14:00	(m) d	(m) 7–10				
Pariana stenolemma	M	protog.	F	(f) 18:00–19:00	(f) n	(f) 10–12	—	néctar-polen	0.5–1.0	AS-253
				(m) 08:00–12:00	(m) d–n	(m) 4–8				
Marantaceae										
Calathea casupito	H	homog.	F	07:00–08:00	d	10	—	néctar	0.5–1.0	AS-221
Calathea sp.	H	homog.	F	06:00–07:00	d	10	—	néctar	0.8–1.0	AS-346
Stromanthe jacquinii	H	homog.	F	09:00–10:00	d	12–14	—	néctar	0.5–1.5	AS-237
Stromanthe tonckat	H	homog.	F	07:30–09:00	d	7	—	néctar	1.0–1.5	AS-206
Musaceae										
Heliconia acuminata	H	homog.	F	04:00–05:30	d	11	—	néctar	2.0–3.0	AS-213
Heliconia aurea	H	homog.	F	05:00–07:00	d	12	—	néctar	1.5–2.0	AS-123
Heliconia bihai	H	homog.	F	05:30–07:00	d	12	—	néctar	1.5–2.0	OH-2154
Heliconia revoluta	H	homog.	F	05:30–07:00	d	12	—	néctar	1.0–2.0	AS-155
Orchidaceae										
Govenia fasciata	H	homog.	F	06:00–12:00	d–n	72	—	néctar	0.3–0.5	AS-153
Govenia utriculata	H	homog.	F	06:00–12:00	d–n	72	—	néctar	0.3–0.5	AS-233
Palmae										
Chamaedorea pinnatifrons	D	—	F	(m) 06:00–12:00	(m) d–n	(m) 48	—	polen	1.5–3.0	AS-149
				(f)	(f) d–n	(f) 96				
Geonoma simplicifrons	M	protan.	F	(m) 05:00–06:00	(m) d	(m) 10	—	néctar-polen	0.5–1.5	AS-158
				(f) 06:00–18:00	(f) d–n	(f) 192				
Geonoma tenuis	M	protan.	F	(m) 06:30–08:00	(m) d	(m) 12	—	néctar-polen	0.5–1.0	AS-172
				(f) 06:00–18:00	(f) d–n	(f) 192				
Socratea sp.	M	protog.	F	(f)	(f) d–n	(f)	—	néctar-polen	10.0–30.0	AS-188
				(m)	(m) d–n	(m)				

TABLA 1. Continuada.

Familia Especie	Sexualidad[1]	Expresión sexual[2]	Unidad de polinización[3]	Hora de antesis	Período de vida floral[4]	Longevidad máxima (h)		Recompensa	Altura de las estructuras reproductivas (m)	Espécimen de referencia[5]
						Flor	Unidad			
Zingiberaceae										
Costus spiralis	H	homog.	F	03:00–05:00	d	12–14	—	néctar	1.5–3.0	AS-169
Renealmia nicolaioides	H	homog.	F	14:00–15:30	d	8	—	néctar	0.1–0.3	AS-207

f = flor femenina o pistilada; m = flor masculina o estaminada.
[1] H = Hermafrodita; M = Monoica; D = Dioica.
[2] homog. = homogama o adicogama; protan. = protandria; protog. = protoginea.
[3] F = Flor; I = Inflorescencia.
[4] d = diurno; n = nocturno.
[5] Colecciones de referencia depositadas en VEN: AS = A. Seres; JS = J. Steyermark; OH = O. Huber.

TABLA 2. Coeficientes de variación ($\bar{X}/S^2$) de la hora de antesis y longevidad de las flores para cada grupo de polinizadores.

Sexualidad Polinizadores	Antesis: Grupo mañana	Antesis: Grupo tarde	Longevidad
Monoicas			
Coleópteros	(f) 0.17	(f) 0.05	(f) 1.28
	(m)0.44	(m)0.23	(m)1.32
Moscas	(f) 0.19	(f) ND	(f) 0.79
	(m) ND	(m)0.00	(m)0.33
Viento	(f) 0.11		(f) 0.17
	(m) 0.10		(m)1.04
Hermafroditas			
Murciélagos	—	0.02	0.12
Aves	0.29	ND	0.26
Abejas	0.18	—	1.07

(f): flor femenina; (m): flor masculina; ND: no disponible.

las flores femeninas fue aproximadamente 19 veces mayor que la de las masculinas, es decir, las flores masculinas vivían de 10 a 12 horas y las femeninas 192 horas en promedio (Tabla 1). Un patrón diferente fue observado en *Pariana stenolemma* Tutin; las flores femeninas abren entre las 18:00 y 19:00 horas y las masculinas entre las 8:00 y 12:00 horas. En este caso, las flores masculinas vivían de 4 a 8 horas y las femeninas de 10 a 12 horas (Tabla 1).

La antesis en las especies anemófilas fue comparativamente menos sincronizada que en las de polinización biótica. Las flores femeninas abrían entre las 6:00 y las 16:00 horas, mientras que las flores masculinas abrían entre las 7:00 y 14:00 horas. La longevidad de las flores femeninas anemofílicas fue de 72 a 96 horas mientras que en las masculinas fue de 7 a 10 horas en Gramineae y Cyperaceae, y de 96 horas en *Chamaedorea pinnatifrons* Jacq.; la relación longevidad femenina : masculina en el primer grupo fue de aproximadamente 7 a 10 y en el segundo grupo de 2.

Los coeficientes de variación de la hora de antesis y la longevidad floral relativo a los distintos mecanismos de polinización están en la Tabla 2. La polinización por murciélagos mostró una alta sincronización en la hora de antesis y longevidad. El grado de variación en la hora de antesis en plantas polinizadas por colibríes fue mayor que en plantas polinizadas por abejas (Tabla 2). La variación en la longevidad en plantas polinizadas por colibríes fue menor que en plantas polinizadas por abejas (Tabla 2). Las plantas melitofílicas presentaron una alta sincronía en la hora de antesis, pero

TABLA 3. Número y porcentaje de especies de agentes visitantes y polinizadores relativo a los grupos taxonómicos.

Orden / Familia	Especies visitantes		Total	
	Visitantes N (%)	Polinizadores N (%)	Visitantes N (%)	Polinizadores N (%)
Apodiformes				
Trochilidae	7 (9.9)	7 (14.6)	7 (9.9)	7 (14.6)
Chiroptera				
Phyllostomidae	3 (4.2)	3 (6.3)	3 (4.2)	3 (6.3)
Coleoptera				
Chrysomelidae	6 (8.5)	6 (12.5)		
Curculionidae	15 (21.1)	11 (22.9)		
Hydrophilidae	1 (1.4)	1 (2.1)		
Nitidulidae	4 (5.6)	1 (2.1)		
Ptilidae	1 (1.4)	1 (2.1)		
Scarabaeidae	4 (5.6)	2 (4.2)		
Staphylinidae	2 (2.8)	2 (4.2)		
No identificadas	1 (1.4)	1 (2.1)	34 (47.9)	25 (52.1)
Dermaptera				
Labiidae	1 (1.4)	0 (0.0)	1 (1.4)	0 (0.0)
Diptera				
Cecidomyiidae	2 (2.8)	2 (4.2)		
Drosophilidae	3 (4.2)	3 (6.3)		
Muscidae	1 (1.4)	0 (0.0)		
Phoridae	2 (2.8)	2 (4.2)		
Sciaridae	1 (1.4)	1 (2.1)		
Syrphidae	1 (1.4)	0 (0.0)		
No identificadas	1 (1.4)	0 (0.0)	11 (15.5)	8 (16.7)
Hymenoptera				
Anthophoridae	2 (2.8)	1 (2.1)		
Apidae	7 (9.9)	2 (4.2)		
Formicidae	1 (1.4)	0 (0.0)		
Halictidae	1 (1.4)	1 (2.1)	11 (15.5)	4 (8.3)
Lepidoptera				
Pieridae	1 (1.4)	0 (0.0)		
Sphingidae	1 (1.4)	0 (0.0)	2 (2.8)	0 (0.0)
Orthoptera				
No identificado	1 (1.4)	0 (0.0)	1 (1.4)	0 (0.0)
Abioticos	1 (1.4)	1 (2.1)	1 (1.4)	1 (2.1)
Total	71 (100.0)	48 (67.6)		

fueron altamente variables en la longevidad. En las especies monoicas, la antesis fue menos variable en comparación a la longevidad de las flores (Tabla 2). La antesis de las flores femeninas fue comparativamente más sincronizada que la de las flores masculinas entre plantas polinizadas por coleópteros y moscas. En contraste, los coeficientes de variación de flores masculinas y femeninas en las plantas anemófilas fueron similares (Tabla 2). La longevidad de las flores masculinas y femeninas en plantas polinizadas por coleópteros presentó la mayor variación en relación al resto de los grupos de plantas. La longevidad de las flores femeninas polinizadas por dípteros presentó una mayor variación en relación a las flores masculinas. En contraste, la longevidad de las flores masculinas polinizadas por el viento fue mucho más variable que la de las flores femeninas.

AGENTES VISITANTES Y POLINIZACION

De un total de 71 agentes visitantes registrados en 33 especies de plantas (Tabla 3), el 67.6% fueron polinizadores efectivos, mientras que el 32.4% fueron

visitantes. Los coleópteros representaron el grupo de visitantes florales más numeroso (Tabla 3), entre los cuales destacaron Curculionidae, Chrysomelidae, Nitidulidae, y Scarabaeidae. El transporte de polen era abundante y frecuentemente sobre todo el cuerpo en Curculionidae, Chrysomelidae, Hydrophilidae, y Staphylinidae, aunque en otros grupos menos numerosos la deposición del polen fue ventral. El transporte dorsal de polen fue aún menos frecuente (Anexo I). El número de especies de plantas visitadas por cada especie de coleóptero (desde 1 hasta 6) fue comparativamente superior en comparación con el resto de los insectos visitantes.

Los himenópteros y dípteros representaron los otros dos grupos más diversos en la muestra examinada (Tabla 3). Entre los dípteros destacaron las Drosophilidae con el mayor número de especies (Tabla 3). Las diferentes especies de dípteros visitaron sólo una especie de planta cada una, y el transporte de polen era generalmente disperso sobre todo el cuerpo. En otros casos eran sólo agentes visitantes (Anexo I). Los himenópteros estaban representados por cuatro familias entre las que destacó Apidae con el mayor número de especies (Tabla 3). Aproximadamente un tercio de las especies de himenópteros fueron agentes polinizadores. En el caso de los polinizadores el polen era frecuentemente transportado en la base de la proboscis y en la parte ventral del cuerpo de manera escasa o dispersa (Anexo I).

Los ortópteros, dermápteros, y lepidópteros fueron los ordenes con el menor número de especies, que sólo actuaron como visitantes sin transportar polen.

Las aves visitantes estaban representadas por siete especies y los murciélagos por tres especies (Tabla 3). Los colibríes transportaban el polen sobre el pico y la base del pico, y los murciélagos sobre la cabeza (Anexo I).

POSICION SISTEMATICA Y MODOS DE POLINIZACION

En la muestra de 33 especies de monocotiledóneas estudiadas hubo una notable correlación entre las Araceae, Cyclanthaceae, Palmae, y una Poaceae, y la polinización por escarabajos. En el caso de las palmas y la gramínea entomófila, muchas veces se combina la polinización por escarabajos y por moscas, mientras que en otros casos son anemófilas, como *Olyra latifolia* L. (Poaceae). Otra tendencia interesante ocurrió entre las Musaceae y Zingiberaceae con la polinización por colibríes. Algunas Marantaceae y Bromeliaceae son también polinizadas por estas aves, e inclusive las Bromeliaceae quirópterofilas son visitadas por colibríes. Las abejas, frecuentemente grandes, polinizaban algunas Marantaceae, Orchidaceae, y Commelinaceae (Anexo 1).

POLINIZACION ENTRE MONOCOTILEDONEAS

La distribución de frecuencia de los modos de polinización de 33 monocotiledóneas mostró una marcada tendencia a ser polinizadas por coleópteros (N = 14; 36.9%), aves (N = 9; 23.7%), y abejas (N = 6; 15.82%). Las plantas polinizadas por moscas (N = 4; 10.6%), viento (N = 3; 7.9%) y murciélagos (N = 2; 5.3%) son grupos menos abundantes.

SEXUALIDAD DE LAS PLANTAS, VARIACION TEMPORAL EN LA MADURACION SEXUAL Y HERCOGAMIA

En 32 especies hay una marcada asociación entre la sexualidad de las plantas y la variación temporal en la maduración de los sexos. Las especies hermafroditas (17) fueron adicógamas, mientras que las monoicas fueron todas dicógamas (15) ($gl = 1$, $\chi^2 = 31.0$; $P < 0.001$). La protogínia fue mas frecuente (40.6%) que la protandria (6.3%). La hercogamia (N = 5; 15.6%) fue menos frecuente que la dicogamia.

El porcentaje de modos de expresión temporal en la maduración sexual relativa a los modos de polinización muestra que las plantas adicógamas son polinizadas por abejas, aves y murciélagos (Tabla 4). En contraste, las plantas protandras y protóginas son polinizadas por escarabajos, viento y moscas. Las especies cantarofílicas son predominantemente protóginas y en menor proporción protandras. Las plantas polinizadas por moscas son protóginas o protandras. Las dos especies anemófilas monoicas son protóginas. Además, la única especie dioica es polinizada por el viento (Tabla 4).

DISCUSION

SEXUALIDAD, DICOGAMIA, Y MODO DE POLINIZACION

En las especies herbáceas del bosque nublado estudiado hay una alta proporción de especies monoicas asociadas a un sistema de dicogamia. La dicogamia reduce la interferencia polen-estigma y actúa como un mecanismo que promueve la fertilización cruzada (Lloyd & Webb, 1986). La proporción de especies monoicas protóginas es considerablemente mayor que la de las protandras, y ambos grupos son polinizados principalmente por escarabajos. La protoginia es esperada en especies cuyos polinizadores ocupan la flor o inflorescencia por largos períodos, así que el polen puede ser depositado en los estigmas a la entrada y removido

TABLA 4. Variación temporal en la maduración sexual relativo a los síndromes de polinización.

Modo de polinización	Adicogamas N (%)	Protoginas N (%)	Protandras N (%)	Dioicas N (%)	Total* N (%)
Coleopteros	—	12 (31.6)	2 (5.3)	—	14 (36.9)
Moscas	—	2 (5.3)	2 (5.3)	—	4 (10.6)
Abejas	6 (15.8)	—	—	—	6 (15.8)
Murcielagos	2 (5.3)	—	—	—	2 (5.3)
Aves	9 (23.7)	—	—	—	9 (23.7)
Viento	—	2 (5.3)	—	1 (2.6)	3 (7.9)

* El total de especies excede el total de 33 especies debido a que cinco especies son polinizadas por dos grupos diferentes de polinizadores.

a la salida (Lloyd & Webb, 1986). La protoginia es frecuente en ciertos grupos taxonómicos de plantas: Annonaceae, Magnoliaceae, Araceae, Cyclanthaceae, y Palmas entre otros grupos (Faegri & van der Pijl, 1979; Thien, 1974; Beach, 1982). La relación entre la polinización por escarabajos y Cyclanthaceae, Araceae, y Arecaceae sugiere que la evolución de la dicogamia está relacionada con ciertos grupos de agentes visitantes en el estrato bajo del bosque nublado. En contraste, las especies polinizadas por aves, abejas, y murciélagos son todas adicógamas y de diferentes familias de plantas, excepto Musaceae y Zingiberaceae que son sólo polinazadas por aves. En algunas de estas plantas la hercógamia promueve la polinización cruzada.

POSICION DE LAS CARGAS DE POLEN

A pesar que la posición de las cargas son determinantes en el proceso de polinización (Feinsinger et al., 1986; Ramírez, 1989), no se encontró una clara distribución en la posición de las cargas de polen sobre el cuerpo de los polinizadores de especies cantarófilas y ornitófilas, en las cuales los períodos de floración estaban solapados (Seres & Ramírez, 1993). De acuerdo con estos resultados, las estrategias de especificidad en el transporte de polen pueden ser alcanzadas a través de la biología floral y otros atributos ecológicos.

ANTESIS Y LONGEVIDAD

La hora de antesis y la longevidad de las flores son particularmente importantes en especies ornitófilas y cantarófilas-miófilas. La variación en la hora de antesis en especies ornitófilas contribuye a la especificidad de los polinizadores. Por ejemplo, la antesis en horas de la tarde en *Renealmia nicolaioides* permite la utilización de los mismos polinizadores de especies de plantas con antesis más temprana. La variación en la hora de antesis en especies cantarófilas y miófilas con polinizadores comunes y períodos de floración solapados muestra una situación mas compleja. El desfasaje en la hora de antesis (mañana-tarde) está asociada a la utilización de diferentes grupos de polinizadores. Cyclanthaceae y Palmae con antesis en horas de la mañana son polinizadas por Curculionidae, y Araceae con antesis en horas de la tarde son polinizadas por Scarabaeidae. Sin embargo, *Cyclanthus bipartitus* (Cyclanthaceae) es polinizado por Scarabaeidae y con una antesis en horas de la tarde (ver también Beach, 1982). Este comportamiento similar al de las especies de Araceae sugiere que hay un efecto notorio del agente específico (grupo taxonómico) de polinización sobre la hora de antesis. Esta asociación reduce el número de plantas cantarófilas que interactuan, pero las plantas polinizadas por Curculionidae y las plantas polinizadas por Scarabaeidae deben evitar la interferencia por otros mecanismos.

Una mayor longevidad puede incrementar el éxito de polinización por el incremento en la oportunidad de fertilización. Frankie et al. (1983) reportaron que las flores adaptadas a la polinización por abejas tienen una longevidad de un día en la mayoría de las especies arbóreas de un bosque seco en Costa Rica. Similarmente, la longevidad de las flores fue de un día en la mayoría de las especies de un arbustal de la Guayana Venezolana (Ramírez et al., 1990) y en un arbustal costero de Jamaica (Percival, 1974). En contraste, la longevidad de las unidades de polinización en muchas especies herbáceas del bosque nublado es superior a un día. En general, nuestros resultados concuerdan con los de Stratton (1989), quien mostró que la longevidad de las flores en plantas herbáceas fue mayor que en arbustos y árboles, así como en especies de bosques nublados. Sin embargo, la mayor longevidad en flores hermafroditas y en flores femeninas tiene diferente significado: (1.) La mayor longevidad en flores e inflorescencias como unidad de polinización en plantas herbáceas del bosque nublado puede estar asociada a la baja predictibilidad

de polinización reportada (Sobrevila & Arroyo, 1982; Ramírez et al., 1990). Gottsberger et al. (1980) relacionaron los polinizadores poco predecibles e inespecíficos y la extensa longevidad floral. La longevidad floral de 72 horas fue observada en orquídeas las cuales recibían visitas muy esporádicas de los agentes polinizadores. (2.) La mayor longevidad en flores femeninas de especies diclinas puede resultar en maximizar el éxito reproductivo femenino (Stratton, 1989). Además, la extensa longevidad de las flores femeninas puede incrementar la probabilidad de polinización y de recibir mayor variedad de genotipos masculinos. Este comportamiento es particularmente importante si consideramos que todas las especies monoicas-dicógamas estudiadas fueron no autógamas (Ramírez & Seres, 1994).

ATRACCION FLORAL

Especies de Dynastinae (Coleoptera, Scarabaeidae), que visitan las flores de *Talauma* y *Philodendron*, consumen partes de raices y órganos subterráneos de los mismos durante su desarrollo larval. Estos últimos son similares en sabor a las flores de estas especies, lo cual parece precondicionar la visita de sus flores cuando alcanzan el estado adulto (Gottsberger, 1986). Las especies de Cyclanthaceae polinizadas por Curculionidae y las especies de Araceae polinizadas por Scarabaeidae podrían incrementar la especificidad de polinización de ésta manera: grupos de coleópteros precondicionados en los estados larvales por la alimentación de frutos y semillas en Cyclanthaceae y tallos y raices en Araceae.

DISPOSICION ESPACIAL

El arreglo vertical de las plantas puede condicionar la trayectoria de vuelo de los insectos, aves y murciélagos, incrementando la probabilidad de polinización (Smith, 1973). Entre las Cyclanthaceae estudiadas, *Asplundia fendleri* Harling, *Evodianthus funifer* (Poit.) Lindm., y *Asplundia moritziana* (Klotzsch) Harling ocupan diferentes posiciones verticales del bosque nublado. Entre las Bromeliaceae polinizadas por murciélagos, las especies de plantas así como sus polinizadores están separadas verticalmente.

Las plantas pueden reducir la interferencia por el aislamiento espacial (Pleasants, 1980). La ubicación de las especies en sub-ambientes del bosque nublado representa otra alternativa que promueve la especificidad de polinización. Por ejemplo, las especies cantarófilas (*Asplundia fendleri*) y quiropterófilas (*Vriesea splendens* (Brongn.) Lem. var. *longibracteata* (Baker) L. B. Smith) crecen exclusivamente en bosque nublado, mientras que *Asplundia* sp. y *Vriesea platynema* Gand. son de bosque nublado de transición (Seres & Ramírez, 1990). Las especies de *Vriesea* son polinizadas por diferentes especies de murciélagos, mientras que las especies de *Asplundia* tienen algunos polinizadores comunes. En este último caso, la distribución de las especies de plantas en sub-ambientes podría reducir la interferencia en el transporte de polen si hay diferentes grupos de polinizadores asociados a determinados sub-ambientes.

CONCLUSION

La distribución de los modos de polinización en el bosque nublado de Rancho Grande está asociada a los grupos taxonómicos de plantas. La biología floral y los diversos modos de polinización promueven la especificidad de polinización y la fertilización cruzada según: (1.) diferencias morfológicas de flores e inflorescencias; (2.) diferentes horas de antesis (ornitofilia y parcialmente cantarofilia); (3.) extensa longevidad floral (melitofilia) y de flores femeninas (cantarofilia y anemofilia); (4.) diferentes polinizadores y períodos de floración (quiropterofilia y algunas cantarófilas); y (5.) la distribución vertical y horizontal de las plantas puede condicionar la especificidad de polinización en especies cantarófilas y quiropterófilas.

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ANEXO 1. Grupos taxonómicos de plantas, visitantes florales, polinizadores, y características de las cargas de polen de 33 especies vegetales.

Familia Especie	Orden Familia Especie	Función	Polen Cantidad	Polen Lugar de transporte	Conclusión
Araceae					
Dieffenbachia sp.	Coleoptera				
	Scarabaeidae				
	Cyclocephala tutilina Burmeister	1	A	V (c, t, a, e)	
	Dermaptera				
	Labiidae				
	Vostox punctipennis (Stal)	2	—	—	
	Diptera				
	Muscidae				
	Mydaea sp.[1]	2	—	—	Cantarófila
Philodendron macroglossum Schott	Coleoptera				
	Scarabaeidae				
	Cyclocephala tutilina Burmeister	1	A	V (c, t, a, e)	Cantarófila
Xanthosoma undipes (Koch) Koch	Coleoptera				
	Nitidulidae				
	Brachypeplus sp.[1]	2	—	—	
	Brachypeplus sp.[2]	2	—	—	
	Macrostela lutea Murray	2	—	—	
	Scarabaeidae				
	Cyclocephala tutilina Burmeister	1	A	V (c, t, a, e)	
	Cyclocephala sexpunctata Castelnau	1	A	V (c, t, a, e)	
	N.I. sp.[1]	2	—	—	
	Dermaptera				
	Labiidae				
	Vostox punctipennis (Stal)	2	—	—	Cantarófila
Bromeliaceae					
Aechmea lasseri L.B. Smith	Apodiformes				
	Trochilidae				
	Coeligena coeligena coeligena (Lesson)	1	A	p	Ornitófila
Pitcairnia altensteinii (Lk., Kl. y Otto) Lem.	Apodiformes				
	Trochilidae				
	Schistes geoffroyi geoffroyi (Bourcier)	3	—	—	
	Sternoclyta cyanopectus (Gould)	1	D	bp, f	

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen: Cantidad	Polen: Lugar de transporte	Conclusión
	Hymenoptera				
	Apidae				
	Trigona fulviventris Guerin	2	D	V (c, t, a, e)	Ornitólfila
Vriesea platynema Gaud.	Apodiformes				
	Trochilidae				
	Sternoclyta cyanopectus (Goud)	2	—	—	
	Chiroptera				
	Phyllostomidae				
	Anoura cultrata	1	A	mi, ic, ip′	
	Anoura geoffroyi geoffroyi	1	A	mi, ic, ip′	
	Hymenoptera				
	Apidae				
	Apis mellifera L.	2	—	—	
	Trigona fulviventris Guerin	2	D	V (a)	
	Lepidoptera				
	Sphingidae				
	N.I. sp.[1]	2	—	—	Quiropterófila
Vriesea splendens (Brongn.) Lem. var. *longibracteata* (Baker) L. B. Smith	Apodiformes				
	Trochilidae				
	Sternoclyta cyanopectus (Gould)	3	—	—	
	Chiroptera				
	Phyllostomidae				
	Anoura caudifer caudifer	1	A	ic, sc′	Quiropterófila
Commelinaceae					
Tradescantia zanonia (L.) H.B.K.	Diptera				
	Syrphidae				
	Leucopodella sp.[1]	2	—	—	
	Hymenoptera				
	Halictidae				
	Chlerogas sp.[1]	1	D	V (t, a, e)	
	Apidae				
	N.I. sp.[1]	2	—	—	Melitófila

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen: Cantidad	Polen: Lugar de transporte	Conclusión
Cyclanthaceae					
Asplundia fendleri Harling	Coleoptera				
	Curculionidae (Erirrhininae)				
	Phyllotrox sp.[1]	1	A	TC	
	Phyllotrox sp.[2]	1	A	TC	
	Phyllotrox sp.[3]	1	A	TC	
	Phyllotrox sp.[4]	1	A	TC	
	Phyllotrox sp.[5]	1	D	TC	
	Hydrochilidae				
	Pelosoma sp.[1]	1	A	TC	
	Scarabaeidae				
	Chlorota terminata Serville	4	—	—	
	Staphylinidae (Aleocharinae)				
	N.I. sp.[1]	1	D	TC	Cantarófila
Asplundia moritziana (Klotzsch) Harling	Coleoptera				
	Curculionidae (Erirrhininae)				
	Phyllotrox sp.[1]	1	A	TC	
	Phyllotrox sp.[3]	1	A	TC	
	Phyllotrox sp.[4]	1	A	TC	
	N.I. sp.[1]	1	D	TC	
	Hydrophilidae				
	Pelosoma sp.[1]	1	A	TC	
	Ptilidae				
	N.I. sp.[1]	1	A	V (c, t, a, e)	
	Staphylinidae (Aleocharinae)				
	N.I. sp.[1]	1	D	TC	Cantarófila
Asplundia sp.	Coleoptera				
	Curculionidae (Erirrhininae)				
	Phyllotrox sp.[1]	1	A	TC	
	Phyllotrox sp.[3]	1	A	TC	
	Hydrophilidae				
	Pelosoma sp.[2]	1	A	TC	
	Ptilidae				
	N.I. sp.[1]	1	E	V (c, t, a, e)	

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen: Cantidad	Polen: Lugar de transporte	Conclusión
	Staphylinidae (Aleocharinae)				
	N.I. sp.[1]	1	D	TC	Cantarófila
Asplundia caput-medusae (Hook. f.) Harling	Coleoptera				
	Curculionidae (Erirrhininae)				
	Phyllotrox sp.[1]	1	A	TC	
	Phyllotrox sp.[2]	1	A	TC	
	Phyllotrox sp.[3]	1	A	TC	
	Hydrophilidae				
	Pelosoma sp.[3]	1	A	TC	
	Staphylinidae (Aleocharinae)				
	N.I. sp.[1]	1	D	TC	Cantarófila
Cyclanthus bipartitus Poit.	Coleoptera				
	Scarabaeidae				
	Cyclocephala tutilina Brumeister	1	A	V (c, t, a, e)	
	Dermaptera				
	Labiidae				
	Vostox punctipennis (Stal)	2	E	V (c, t, a)	Cantarófila
Evodianthus funifer (Poit.) Lindm.	Coleoptera				
	Curculionidae (Erirrhininae)				
	Phyllotrox sp.[1]	1	A	TC	
	Phyllotrox sp.[2]	1	A	TC	
	Phyllotrox sp.[3]	1	A	TC	
	Phyllotrox sp.[4]	1	A	TC	
	N.I. sp.[2]	1	D	TC	
	Hydrophilidae				
	Pelosoma sp.[4]	1	A	TC	
	Ptilidae				
	N.I. sp.[1]	1	E	V (c, t, a, e)	
	Scarabaeidae				
	Chlorota terminata Serville	3	—	—	
	Staphylinidae (Aleocarinae)				
	N.I. sp.[1]	1	D	TC	Cantarófila
Cyperaceae					
Scleria latifolia Sw.	Viento	1	—	—	Anemófila

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen Cantidad	Polen Lugar de transporte	Conclusión
Gramineae (Poaceae)					
Olyra latifolia L.	Viento	1	—	—	Anemófila
Pariana stenolemma Tutin	Coleoptera				
	Chrysomelidae				
	Sanariana sp.[1]	1	D	TC	
	Sanariana sp.[2]	1	D	a, e	
	Sanariana sp.[3]	1	D	a, e	
	Curculionidae (Barinae)				
	Centrinaspis sp.[2]	1	A	TC	
	Centrinaspis sp.[3]	1	A	TC	
	Ptilidae				
	N.I. sp.[1]	1	E	V (c, t, a, e)	
	Diptera				
	Cecidomyiidae				
	Clinodiplosis sp.[1]	1	D	TC	
	Clinodiplosis sp.[2]	1	D	TC	
	Phoridae				
	N.I. sp.[1]	1	D	TC	
	N.I. sp.[2]	1	D	TC	
	Hymenoptera				
	Apidae	2	—	—	Cantarófila-Miófila
	Apis mellifera L.				
Marantaceae					
Calanthea casupito (Jacq.) Presl	Hymenoptera				
	Apidae				
	Eulaema speciosa (Mocsary)	1	E	pb	Melitófila
Calathea sp.	Hymenoptera				
	Apidae				
	Eulaema speciosa (Mocsary)	1	E	pb	Melitófila
Stromanthe jacquinii (Roem. & Schult.) Kennedy y Nicolson	Apodiformes				
	Trochilidae				
	Adelomyia melanogenys aeneosticta Simon	1	E	bp	
	Ocreatus underwoodii polystictus Todd	1	E	bp	
	Schistes geoffroyi geoffroyi (Bourcier)	1	E	bp	

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen: Cantidad	Polen: Lugar de transporte	Conclusión
	Coleoptera				
	Curculionidae				
	Cholus brunnirostris Chevr.	4	—	—	
	Hymenoptera				
	Anthophoridae				
	Rhathymus sp.[1]	1	E	pb	
	Apidae				
	Trigona fulviventris Guerin	2	—	—	Ornitófila
Stromanthe tonckat (Aubl.)	Hymenoptera				
	Anthophoridae				
	Osiris analis	2	—	—	
	Rhathymus sp.[1]	1	E	pb	
	Apidae				
	Bombus sp.[1]	1	E	pb	
	Paratetrapedia sp.[1]	2	—	—	
	N.I. sp.[1]	2	—	—	
	Lepidoptera				
	Pieridae				
	Dismorphia crisia foedora (Lucas)	2	—	—	Melitófila
Musaceae					
Heliconia acuminata L.C. Richard	Apodiformes				
	Trochilidae				
	Colibri delphinae (Lesson)	1	E	p	
	Schistes geoffroyi geoffroyi (Bourcier)	3	—	—	
	Sternoclyta cyanopectus (Gould)	1	D	bp, f	Ornitófila
Heliconia aurea Rodriguez	Apodiformes				
	Trochilidae				
	Sternoclyta cyanopectus (Gould)	1	D	bp, f	Ornitófila
Heliconia bihai (L.) L.	Apodiformes				
	Trochilidae				
	Sternoclyta cyanopectus (Gould)	1	A	bp, f	Ornitófila
Heliconia revoluta (Griggs) Standley	Apodiformes				
	Trochilidae				

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen: Cantidad	Polen: Lugar de transporte	Conclusión
	Schistes geoffroyi geoffroyi (Bourcier)	3	—	—	
	Sternoclyta cyanopectus (Gould)	1	A	bp, f	
	Hymenoptera				
	Apidae				
	Eulaema seabrai Mour	2	E	e	Ornitófila
Orchidaceae					
Govenia fasciata Lindl.	Hymenoptera				
	Apidae				
	Eulaema speciosa (Mocsary)	1	E	c	Melitófila
Govenia utriculata (Sw.) Lindl.	Hymenoptera				
	Apidae				
	Eulaema speciosa (Mocsary)	1	E	c	Melitófila
Palmae (Arecaceae)					
Chamaedorea pinnatifrons (Jacq.) Oerst.	Viento	1	—	—	
	Visitan flores femeninas:				
	Coleoptera				
	Chrysomelidae				
	Sanariana sp.[4]	1	D	TC	
	Visitan flores masculinas:				
	Coleoptera				
	Chrysomelidae				
	Monolepta sp.[1]	2	D	TC	
	Sanariana sp.[4]	1	D	TC	
	Curculionidae (Cholinae)				
	Cholus brunnirostris Chevr.	2	D	TC	
	Cholus sp.[1]	2	D	V (c, t, a, e)	
	Diptera				
	N.I. sp.[1]	2	E	e	
	Hymenoptera				
	Apidae				
	Trigona fulviventris Guerin	2	—	—	
	Orthoptera				
	N.I. sp.[1]	2	D	TC	Anemófila-Cantarófila

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen: Cantidad	Polen: Lugar de transporte	Conclusión
Geonoma simplicifrons Willd.	Coleoptera				
	Chrysomelidae				
	Cephaloleia aff. *lata* Baly	1	E	e	
	Monolepta sp.[1]	1	D	a, e	
	Sanariana sp.[1]	1	D	TC	
	Sanariana sp.[4]	1	D	TC	
	Curculionidae (Barinae)				
	Centrinaspis sp.[1]	1	D	TC	
	Centrinaspis sp.[4]	1	D	D' (c, t, a, e)	
	Cholus sp.[1] (Cholinae)	2	—	—	
	Phyllotrox sp.[5] (Erirrhininae)	1	D	TC	
	Diptera				
	Drosophilidae				
	N.I. sp.[1]	1	D	TC	Cantarófila-
	N.I. sp.[2]	1	D	D' (c)	Miófila
Geonoma tenuis Burret	Coleoptera				
	Curculinonidae (Barinae)				
	Centrinaspis sp.[1]	1	D	TC	
	N.I. sp.[1]	1	D	D' (c, t, a)	
	Diptera				
	Sciaridae				
	Bradysia sp.[1]	1	D	a, e	
	Hymenoptera				
	Formicidae	2	—	—	Cantarófila-
	Crematogaster nigropilosa Mayr				Miófila
Socratea sp.	Coleoptera				
	Curculionidae				
	N.I. sp.[3]	2	D	D' (c, t, a)	
	N.I. sp.[4]	2	D	D' (c, t, a)	
	Nitidulidae				
	Mystrops aff. *fryi* Grouvelle	1	D	V (c, t, a, e)	
	Scarabaeidae				
	Cyclocephala sexpunctata Castelnau	2	—	—	

ANEXO 1. Continuado.

Familia Especie	Orden Familia Especie	Función	Polen: Cantidad	Polen: Lugar de transporte	Conclusión
	Staphylinidae (Omaliinae)				
	N.I. sp.[2]	1	D	TC	
	Diptera				
	Drosophilidae				
	N.I. sp.[3]	1	D	TC	
	Hymenoptera				
	Apidae				
	Apis mellifera var. *sentellata* Lat.	2	D	V (c, t, a, e)	Cantarófila-Miófila
Zingiberaceae					
Costus spiralis (Jacq.) Roscoe	Apodiformes				
	Trochilidae				
	Coeligena coeligena coeligena (Lesson)	1	A	sp, bp	
	Sternoclyta cyanopectus (Gould)	1	A	bp	Ornitófila
Renealmia nicolaioides Loes.	Apodiformes				
	Trochilidae				
	Coeligena coeligena coeligena (Lesson)	1	A	sp, bp	
	Phaetornis longuemareus ignobilis Todd	1	A	p	
	Sternoclyta cyanopectus (Gould)	1	A	bp	Ornitófila

N.I. = no identificado; 1 = polinizador; 2 = visitante; 3 = perforador de flores; 4 = consume partes florales; E = escaso; A = abundante; D = disperso; V = ventral; D′ = dorsal; TC = todo el cuerpo; t = torax; e = extremidades; c′ = cuello; c = cabeza; m = mandíbula; p = pico; pb = proboscis; a = abdomen; s = superior; i = inferior; b = base; f = frente; p′ = pecho

REVISION DEL GRUPO RACEMOSA DEL GENERO *PASPALUM* (POACEAE: PANICOIDEAE: PANICEAE)[1]

Osvaldo Morrone,[2] *Fernando O. Zuloaga,*[2] *y Eduino Carbonó*[3]

Resumen

En el presente tratamiento se consideran 10 especies del grupo Racemosa del género *Paspalum*, incluyendo dos nuevas especies, *Paspalum rugulosum* Morrone & Zuloaga y *P. soukupii* Carbonó. El grupo Racemosa se caracteriza por incluir plantas anuales que habitan en lugares húmedos hasta los 4600 m de altitud, las que poseen las siguientes características: cañas decumbentes, arraigadas y ramificadas en los nudos inferiores, láminas planas, ovado-lanceoladas, lanceoladas a linear-lanceoladas, inflorescencias con pocos a numerosos racimos decíduos, raquis de los racimos foliáceo, llevando espiguillas uni o biseriadas, con gluma superior o sin la misma y antecio superior cartilaginoso o crustáceo. Las especies de Racemosa son Kranz, del tipo MS, con características células distintivas Kranz en el mesófilo. Se consideran las afinidades de Racemosa con otros grupos de *Paspalum* y se incluye una clave de las especies, descripción anatómica del grupo, fotografías, descripciones morfológicas, y mapas de distribución de todas las especies.

Abstract

Ten species of group Racemosa of genus *Paspalum* are treated in this work, including two new species, *Paspalum rugulosum* Morrone & Zuloaga and *P. soukupii* Carbonó. Group Racemosa is defined as annual plants of medium to high altitudes that inhabit moist places and have the following characteristics: culms decumbent, rooting and branching at the lower nodes, blades flat, ovate-lanceolate, lanceolate to linear-lanceolate, inflorescences with several to many deciduous racemes, rachis of the racemes foliaceous, spikelets uniseriate or biseriate, with or without an upper glume and upper anthecium cartilaginous or crustaceous. Anatomically the species of group Racemosa are characterized as being Kranz, MS type, with peculiar distinctive Kranz cells in the mesophyll. Relationships of this group with other infrageneric taxa of *Paspalum* are discussed. A key to the species, as well as an anatomical description of the group and photomicrographs, morphological description of the species, and distribution maps are presented.

Chase (1929) estableció al grupo Dissecta de *Paspalum* por incluir especies anuales o perennes, con inflorescencias con pocos a muchos racimos, raquis de los racimos foliáceo, ancho y espiguillas comúnmente glabras, con antecio superior pajizo. Chase incluyó dentro de Dissecta plantas acuáticas o subacuáticas o de lugares húmedos y que crecen en bordes de cursos de agua. Chase (1929) basó este grupo en la subsección *Pseudoceresia* de Bentham (1883); este autor consideró en esta subsección a *Paspalum stoloniferum* Bosc (= *P. racemosum* Lam.) y *P. repens* Berg. Chase (1929) trató dentro de Dissecta a *P. dissectum* (L.) L., *P. serratum* Hitchc. & Chase, *P. acuminatum* Raddi, *P. repens*, *P. longicuspe* Nash, entre las especies perennes, y *P. prostratum* Scribn. & Merr., *P. candidum* (Humb. & Bonpl. ex Flüggé) Kunth, *P. scabrum* Scribn., *P. racemosum*, y *P. crassum* Chase entre las especies anuales. Posteriormente Chase (ined.) incluyó a *P. crassum* en el grupo Crassa.

El análisis de las especies de Dissecta sensu lato permitió determinar que existen una serie de caracteres exomorfológicos e histofoliares que permiten diferenciar las especies de Dissecta sensu stricto y las que aquí se incluyen en el grupo Racemosa. En el presente tratamiento se establecen los caracteres distintivos de ambos grupos y

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se relaciona a Racemosa con el grupo Bonplandiana. Cabe destacar que en esta revisión no se ha buscado, al agrupar los taxones en el grupo Racemosa, establecer categorías taxonómicas a nivel infragenérico.

Materiales y Metodos

Para el estudio histofoliar se obtuvieron cortes transversales y epidermis a la altura del tercio medio de la penúltima lámina de la innovación fértil. Se utilizó material proveniente de ejemplares de herbario, previamente tratado con Contrad 70 (Schmid & Turner, 1977) durante 24 a 48 horas a 20°C, o material fresco fijado en FAA. Los cortes transversales se hicieron a mano alzada, previo tratamiento con HF al 5% durante 24 horas. Para la obtención de las epidermis se siguió el método de Metcalfe (1960).

Los cortes fueron coloreados con azul de metileno al 1% y con safranina al 1% en alcohol 80° o con safranina-alcian blue y montados en gelatina-glicerina.

Para la observación de las células clorenquimáticas se realizaron macerados siguiendo el método de Jeffrey (Sass, 1940). Para la identificación de los cuerpos de sílice y células suberosas se utilizó respectivamente fenol líquido (Metcalfe, 1960) y Sudán III (Sass, 1940). La determinación de los plástidos de almidón, y su distribución, se realizó mediante unas gotas de solución iodo-iodurada (Sass, 1940).

Para las descripciones histofoliares se adoptó la terminología propuesta por Ellis (1976, 1979).

Las observaciones anatómicas fueron hechas con un microscopio fotónico Wild M20 con cámara de dibujo. Las disecciones fueron estudiadas con un microscopio estereoscópico Wild M5 con cámara de dibujo.

El estudio exomorfológico fue realizado sobre la base de materiales pertenecientes a los siguientes herbarios: B, BAA, COL, K, LIL, LPB, M, MO, P, SI, UTMC, USM, US, citados conforme a las siglas que figuran en Holmgren et al. (1990). Dentro del material examinado de cada especie sólo se citaron ejemplares representativos de cada país; una lista completa de los especímenes estudiados se encuentra ordenada alfabéticamente por coleccionista al final del texto.

Las fotomicrografías fueron tomadas con un equipo automático Nikon FXA, con cámara fotográfica DX-DB2 35 mm, y la película utilizada Kodak T-MAX de 100 ASA.

Para la obtención de fotomicrografía de epidermis abaxiales de la lemma y pálea del antecio superior se empleó un microscopio electrónico de barrido Jeol JSM-25 SII, perteneciente a la Facultad de Odontología (Universidad Nacional de Buenos Aires, Argentina).

Con un asterisco (*) se señalan en la cita de materiales examinados los ejemplares empleados en el estudio histofoliar. Con dos astericos (**) se indican aquellos especímenes utilizados en el análisis de la lemma y pálea superior.

Caracteres Morfologicos y Taxonomicos

HABITO

El grupo Racemosa incluye especies anuales, con cañas decumbentes y arraigadas en los nudos inferiores, luego erectas; excepcionalmente se presentan plantas arrosetadas en *Paspalum pygmaeum* Hack. Las lígulas son membranáceas y las láminas son planas, ovado-lanceoladas, lanceoladas a linear-lanceoladas, de base redondeada a cordada, angostada y pseudopeciolada en *P. reclinatum* Chase y *P. rugulosum* Morrone & Zuloaga.

INFLORESCENCIA

Un importante carácter que distingue al grupo Racemosa es la presencia de inflorescencias con el raquis de los racimos foliáceo y caedizo a la madurez, cayendo el mismo junto con las espiguillas. Asimismo dentro del grupo se diferencian especies con espiguillas uni o biseriadas y con el raquis terminando en una espiguilla o en una prolongación estéril; se observaron espiguillas uniseriadas en *Paspalum candidum*, *P. soukupii* Carbonó, y *P. prostratum* (ocasionalmente en 2 series en esta última especie), y espiguillas biseriadas en *P. flavum*, *P. pygmaeum*, *P. racemosum*, *P. reclinatum*, *P. rugulosum*, *P. sodiroanum* Hack., y *P. penicillatum* Hook. f.; ocasionalmente se presentan espiguillas uniseriadas en esta última especie. Los racimos terminan en una espiguilla desarrollada en *P. flavum* J. Presl, *P. racemosum*, *P. reclinatum* (rara vez termina en esta especie en una prolongación estéril), y *P. rugulosum*; a su vez el racimo termina en una prolongación estéril en *P. soukupii*, *P. candidum*, *P. prostratum*, *P. pygmaeum*, y *P. penicillatum*. En *P. sodiroanum* el racimo puede culminar indistintamente en una espiguilla o en una prolongación estéril, variando este carácter dentro de la misma inflorescencia.

Los racimos son arqueados en *Paspalum flavum* y rectos en el resto de las especies analizadas. El número de racimos es variable, desde 2 a 6 en *P. pygmaeum* hasta cerca de 60 en *P. flavum* y *P. racemosum*.

ESPIGUILLA

Las espiguillas son uniformemente plano-convexas y existen en las mismas una serie de caracteres que discriminan diferentes especies dentro del grupo:

Disposición de las brácteas. En *P. flavum*, *P. racemosum*, y *P. sodiroanum* las espiguillas maduras son abiertas en su extremidad, hallándose la gluma superior y la lemma inferior separadas hacia el ápice. En el resto de las especies examinadas las espiguillas están cerradas en el ápice.

Textura de las brácteas. La superficie de la gluma superior y la lemma inferior varía de finamente papilosa en *P. flavum* a transverso-rugosa y fuertemente papilosa en *P. racemosum*. En *P. rugulosum* la gluma superior es fuertemente corrugada a lo largo de los nervios laterales y no papilosa. En el resto de las especies incluidas en Racemosa la superficie de las brácteas es lisa y no papilosa.

Reducción de las brácteas. La gluma inferior falta en todas las especies del grupo Racemosa.

La gluma superior está ausente en *P. candidum* y *P. soukupii* y presente en el resto de las especies; dentro de estas últimas la gluma superior supera en largo al antecio superior en *P. flavum*, *P. racemosum*, *P. reclinatum*, *P. rugulosum*, y *P. sodiroanum*, siendo del mismo largo en las restantes especies.

La lemma inferior es más larga que el antecio superior en *P. flavum*, *P. racemosum*, *P. reclinatum*, *P. rugulosum*, y *P. sodiroanum* y posee el mismo largo que el antecio superior en *P. candidum*, *P. prostratum*, *P. pygmaeum*, y *P. penicillatum*. La lemma inferior está ausente en *P. soukupii*.

ANTECIO SUPERIOR

Textura. La lemma y la pálea presentes en las especies del grupo Racemosa poseen el dorso liso, estando la superficie del antecio superior compuesta de células largas rectangulares, más de tres veces más largas que anchas, con las paredes anticlinales longitudinales onduladas y las transversales rectas. La lemma posee los bordes enrollados y cubre los ⅔ de la superficie de la pálea, encerrando el ápice de la misma.

El antecio superior es crustáceo en *P. flavum*, *P. penicillatum*, *P. prostratum*, *P. pygmaeum*, *P. reclinatum*, *P. racemosum*, y *P. sodiroanum*. Posee una textura más cartilaginosa, menos endurecida, en *P. candidum*, *P. soukupii*, y *P. rugulosum*.

Ornamentación (Figs. 1, 2). Sobre la epidermis abaxial se observan papilas, cuerpos de sílice o micropelos. Las papilas son simples, una por célula, excéntricas, próximas a la pared transversal distal. Están distribuidas regularmente en hileras longitudinales sobre la lemma y la pálea en *Paspalum candidum*, *P. penicillatum*, *P. prostratum*, *P. pygmaeum*, *P. sodiroanum*, y *P. soukupii*. Las papilas se presentan únicamente sobre la pálea en *P. reclinatum* y *P. rugulosum* y están ausentes en *P. flavum* y *P. racemosum*.

Los cuerpos de sílice son halteriformes a ligeramente cruciformes, exfoliados, con los ejes equidimensionales, dispuestos hacia el ápice de la lemma y pálea en *P. candidum*, *P. flavum*, *P. racemosum*, *P. reclinatum*, *P. rugulosum*, y *P. soukupii*. Se hallan sólo presentes en el ápice de la lemma en *P. penicillatum* y *P. pygmaeum* y están ausentes sobre la lemma y pálea en *P. prostratum* y *P. sodiroanum*.

Se observaron micropelos 2-celulares, fusiformes en *P. candidum*, *P. prostratum*, *P. sodiroanum*, y *P. soukupii*, distinguiéndose *P. candidum* y *P. soukupii* por tener micropelos cuya célula ápical es igual a 2 veces más larga que la célula basal, estando los micropelos distribuidos sobre la pálea junto a los bordes de la lemma. *Paspalum prostratum* y *P. sodiroanum* tienen micropelos con la célula distal pequeña, ⅓ del largo de la célula basal.

Desarticulación del antecio: El antecio superior desarticula antes que las brácteas en *P. flavum*, *P. racemosum*, y *P. sodiroanum* y cae en conjunto con el resto de las brácteas en las restantes especies.

Desarticulación temprana del antecio superior ha sido previamente señalada en la tribu Paniceae en *Axonopus*, *Brachiaria*, *Echinolaena*, *Ichnanthus*, *Melinis*, *Panicum*, *Pennisetum*, *Rhynchelytrum*, *Sacciolepis*, *Tricholaena*, y *Yakirra* (Lazarides & Webster, 1984; Silberbauer-Gottsberger, 1984; Davidse, 1987; Webster, 1987; Zuloaga, 1987; Morrone & Zuloaga, 1992).

DISTRIBUCION Y ECOLOGIA

Las especies del grupo Racemosa se encuentran desde México hasta el noroeste de la República Argentina. La especie con distribución más amplia es *Paspalum candidum*, la que se encuentra desde México hasta la Argentina. La mayor concentración de especies se encuentra desde Colombia, Ecuador, y Venezuela hasta Perú y Bolivia, donde se hallan *P. penicillatum*, *P. racemosum*, *P. reclinatum*, *P. rugulosum* (endémica de Ecuador), *P. sodiroanum*, y *P. soukupii* (endémica del Perú).

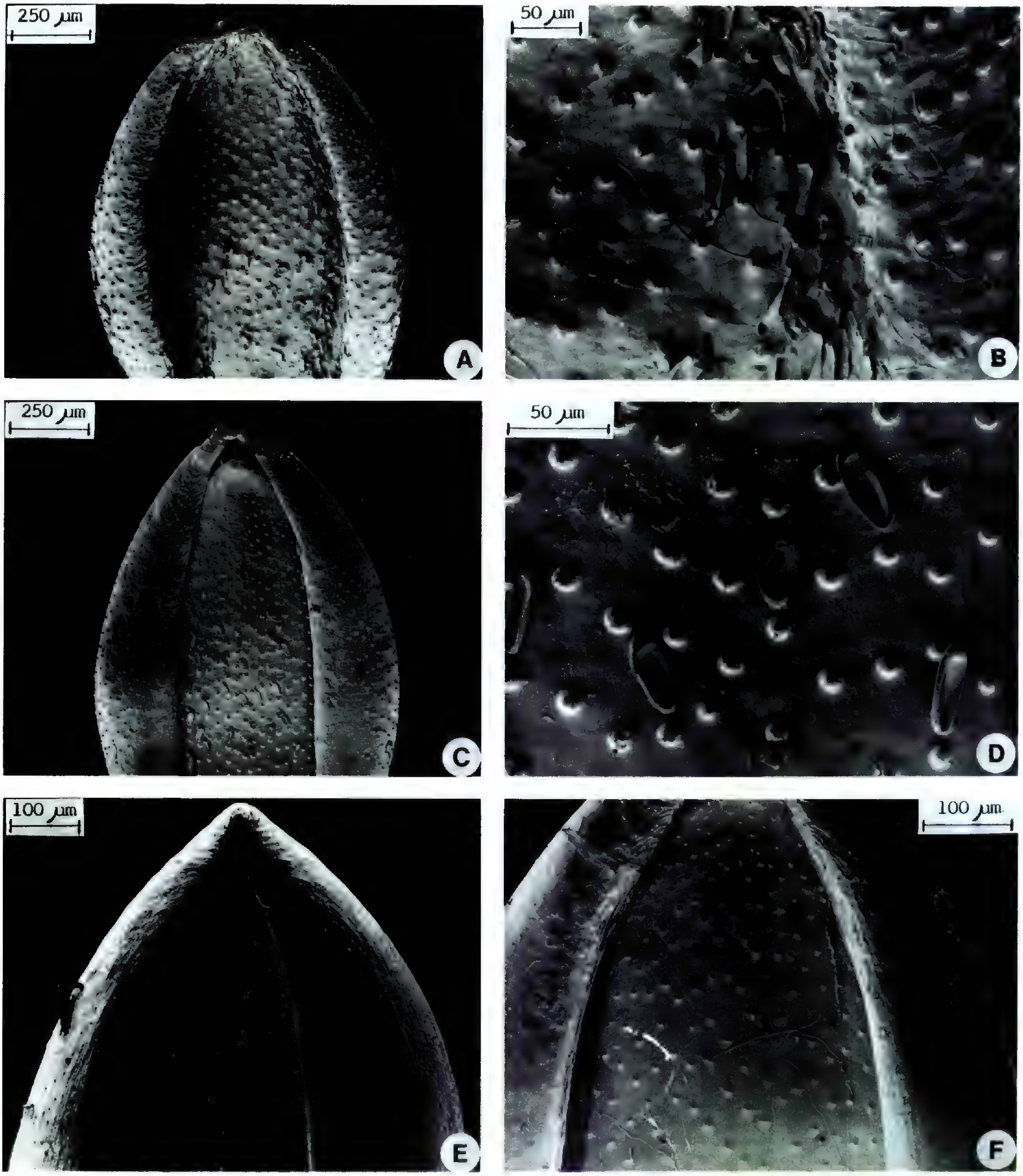


FIGURA 1. Fotomicrografías de antecios superiores de *Paspalum*. A, B, *Paspalum sodiroanum* Hack. (de *Sodiro s.n.*).—A. Antecio superior, visto del lado de la pálea.—B. Detalle de pálea y borde de la lemma mostrando papilas y micropelos bicelulares. C, D, *Paspalum prostratum* Scribn. & Merr. (de *García-Barriga 7836*).—C. Antecio superior, visto del lado de la pálea.—D. Detalle de la superificie de la pálea con papilas y micropelos bicelulares. E, F, *Paspalum pygmaeum* Hack. (E de *Venturi 8392*, F de *Tamayo 1859*).—E. Apice del antecio superior visto del lado de la pálea.—F. Detalle de superficie de la pálea y bordes de la lemma.

En Mesoamérica habitan *P. candidum*, *P. prostratum*, *P. pygmaeum*, y *P. reclinatum*, mientras que a la Argentina sólo llegan *P. candidum* y *P. pygmaeum* y a Chile *P. flavum* y *P. candidum*. *Paspalum prostratum* y *P. pygmaeum* se distinguen por tener distribución disyunta, hallándose *P. pygmaeum* en Colombia, Venezuela, Perú, Bolivia, y la Argentina y ocasionalmente en México (Distrito Federal y en el estado de Morelos); *P. prostratum* habita en México, Venezuela, Colombia, Ecuador, Perú, y Bolivia.

Las especies de Racemosa crecen comúnmente

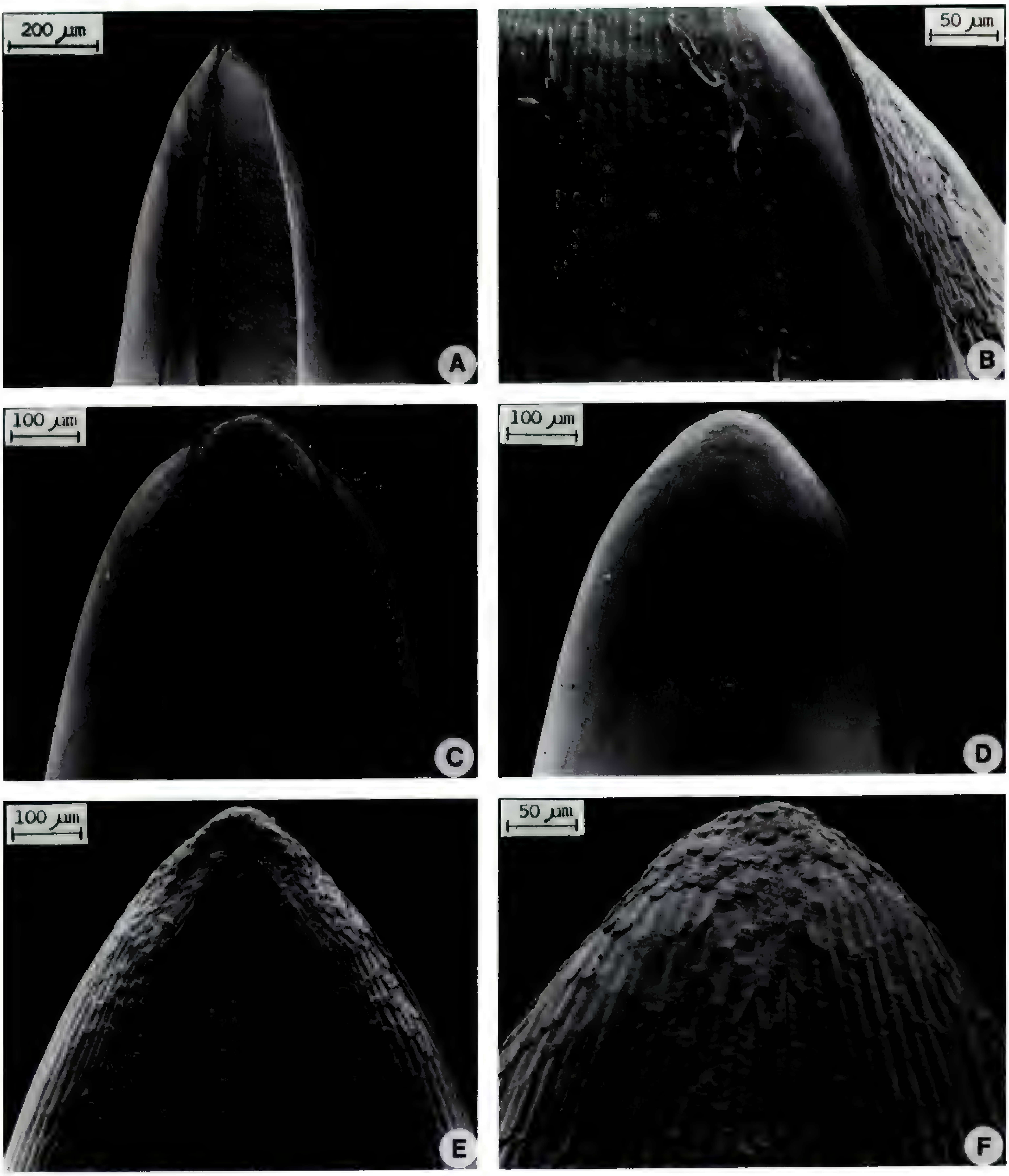


FIGURA 2. Fotomicrografías de antecios superiores de *Paspalum*. A, B, *Paspalum candidum* Humb. & Bonpl. ex Flüggé (de *Hitchcock 20723*).—A. Antecio superior, visto del lado de la pálea.—B. Detalle de pálea y borde de la lemma mostrando papilas y micropelos. C, D, *Paspalum flavum* J. Presl (de *Weberbauer 5707*).—C. Antecio superior, visto del lado de la pálea.—D. Detalle de la lemma. E, F, *Paspalum reclinatum* Chase (de *Pérez Arbeláez & Cuatrecasas 6211*).—E. Antecio superior, visto del lado de la pálea.—F. Apice de la lemma con cuerpos de sílice.

en laderas de montaña, sobre suelos húmedos y pedregosos o en lugares sombríos en bordes de selvas nubladas o bosques; menos frecuentemente se hallan en bordes de ríos. Se encuentran normalmente desde alturas medias a grandes altitudes, entre 1500 y 4000 m, llegando hasta los 4600 m *P. candidum* y *P. pygmaeum*. Crecen ocasionalmente hasta el nivel del mar ejemplares de *P. candidum*, *P. flavum*, *P. penicillatum*, y *P. racemosum*, y se examinaron especímenes aislados de páramos de Colombia y Venezuela de *P. prostratum* y *P. pygmaeum*.

NUMEROS CROMOSOMICOS

Paspalum candidum $n = 10$ (Davidse & Pohl, 1974); $n = 30$ (Davidse & Pohl, 1972); $2n = 20$ (Pohl & Davidse, 1971, bajo *P. scabrum*); $2n = 60$ (Pohl & Davidse, 1971).

Paspalum prostratum $n = 10$ (Davidse & Pohl, 1974); $2n = 60$ (Pohl & Davidse, 1971); $2n = 40$ (Reeder, 1968).

Paspalum reclinatum $n = 30$ (Davidse & Pohl, 1978); $2n = 60$ (Pohl & Davidse, 1971, bajo *P. prostratum*).

ANATOMIA FOLIAR (Fig. 3)

Los caracteres anatómicos de las especies del grupo Racemosa de *Paspalum* permiten inferir que las mismas son C_4 por tener una vaina Kranz con cloroplastos especializados alrededor de los haces vasculares y por poseer células distintivas Kranz aisladas en el transcorte. La presencia de células distintivas Kranz representa una anatomía atípica dentro de las especies C_4, pues existe en estas especies una distancia mayor de 4 células clorenquimáticas entre haces vasculares contíguos, de acuerdo al criterio establecido por Hattersley & Watson (1975). Sin embargo Hattersley & Watson consideran que en este caso particular se debe tomar en consideración la distancia entre haces vasculares y las células distintivas Kranz que es de 1–2 células, rango que corresponde a plantas C_4.

A su vez las especies de Racemosa son C_4 del subtipo MS (XyMS-) (Hattersley & Watson, 1976; Brown, 1977; Hattersley, 1992), caracterizado por tener una única vaina alrededor de los haces vasculares en contacto con los vasos de metaxilema. La presencia de los caracteres previamente mencionados nos permite inferir que las especies analizadas utilizan la vía fotosintética C_4, subtipo NADP-me (Hattersley & Watson, 1976; Brown, 1977).

Las especies de Racemosa poseen una anatomía foliar marcadamente homogénea, con pequeñas diferencias entre las especies que son detalladas en la siguiente descripción general:

CARACTERES HISTOFOLIARES EN CORTE TRANSVERSAL

Transcorte: plano a ligeramente en forma de "V" muy abierto, con las semiláminas simétricas, derechas, delgadas, de 75–170 µm de espesor; cara adaxial y abaxial sin zonas costales y surcos desarrollados. *Haz vascular medio:* estructuralmente distinguible, asociado a un número variable de células parenquimáticas incoloras, con 1 haz vascular de primer orden solitario (en *P. prostratum*) o acompañado por 2–6 haces vasculares de segundo orden y 2–3 haces vasculares de tercer orden o con 1 haz vascular de primer orden, estructuralmente indistinguible de los restantes de la semilámina (en *P. pygmaeum*, *P. rugulosum*, y *P. soukupii*). *Distribución de los haces vasculares:* (2–)4–9 haces vasculares de segundo orden y 2–5 haces vasculares de tercer orden entre haces vasculares de primer orden contiguos, todos equidistantes de ambas epidermis; haces vasculares de tercer orden usualmente localizados por debajo de las células buliformes. *Estructura de los haces vasculares:* haces vasculares de primer orden de contorno elíptico a oval, con vasos de metaxilema más anchos a ligeramente iguales que las células de la vaina Kranz, en contacto directo con ella, de contorno angular, con las paredes ligeramente engrosadas; protoxilema presente; tejido floemático adjunto a la vaina Kranz; haz vascular de segundo orden de contorno elíptico (ausentes en *P. prostratum*), con xilema y floema distinguibles; haces vasculares de tercer orden de contorno angular, con xilema y floema indistinguible. *Vainas de los haces:* haces vasculares de primer, segundo, y tercer orden completamente rodeados por una simple vaina mestomática Kranz, compuesta de células infladas, globosas a reniformes, de paredes ligeramente engrosadas; cloroplastos especializados presentes, llenando completamente el lumen celular o localizados junto a la pared tangencial externa; extensiones de la vaina Kranz ausentes. *Células distintivas Kranz:* subglobosas, de paredes más engrosadas que las células clorenquimáticas adyacentes, con cloroplastos especializados, presentes en el mesófilo por debajo de las células buliformes, equidistantes de ambas epidermis, solitarias, menos frecuentes en pares, no asociadas a tejido vascular; en vista paradermal se disponen en hileras longitudinales paralelas entre los haces vasculares, conectadas entre sí y a los haces vasculares por venillas transversales. *Esclerénquima:* pobremente desarrollado, en grupos discontinuos, en contacto o no con los haces vasculares; margen de la lámina con esclerénquima submarginal. *Mesófilo:* clorénquima compacto, con escasos espacios intercelulares, irregularmente radiado, homogéneo, con células pequeñas, regulares en forma; 1–2 células clorenquimáticas entre células distintivas Kranz y haces vasculares. "Arm cells" ausentes. Células fusoides ausentes. Células parenquimáticas incoloras ausentes. *Células epidérmicas adaxiales:* células buliformes en grupos extensivos, regulares, no asociadas a células parenquimáticas incoloras, compuestas por 8–12 células infladas, con la pared tangencial externa ligeramente arqueada, localizadas sobre los haces vasculares de

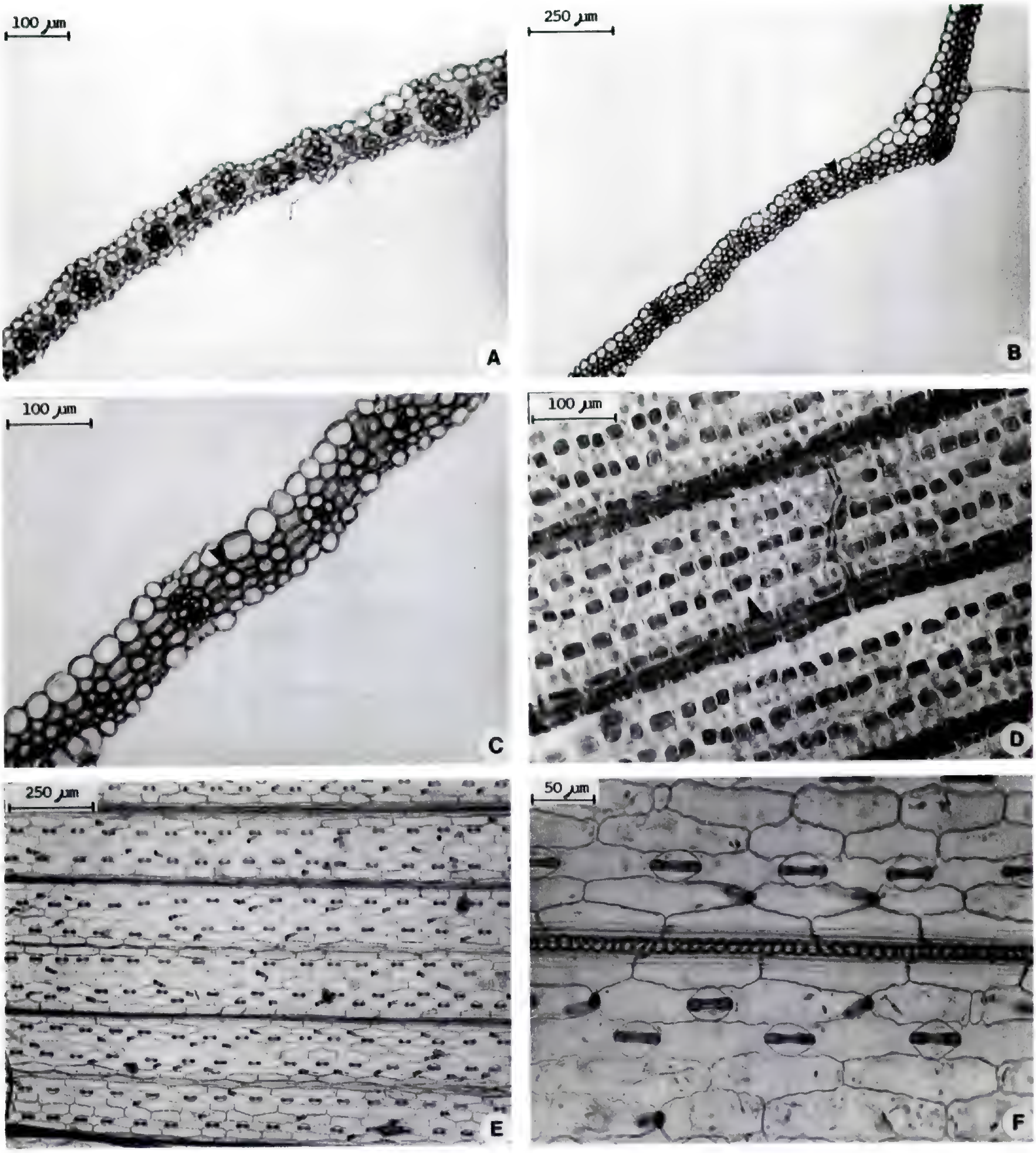


FIGURA 3. A. *Paspalum rugulosum.* Detalle de una porción de la semilámina en transcorte, células distintivas Kranz señaladas con una flecha. B, C. *Paspalum candidum.*—B. Detalle de una porción de la semilámina en transcorte, con células distintivas Kranz.—C. Detalle de transcorte con haces vasculares de primer y tercer orden, células distintivas Kranz señaladas con una flecha.—D. *Paspalum flavum.* Vista paradermal, células distintivas Kranz señaladas con una flecha. E, F. *Paspalum sodiroanum.*—E. Epidermis abaxial.—F. Detalle de E. (A de *Peterson et al. 9249*; B, C de *Deginani y Cialdella 101*; D de *Asplund 13787*; E, F de *Franco et al. 2482.*)

tercer orden, ocupando menos de ⅓ del ancho del transcorte. Células epidérmicas grandes, con la pared externa papilosa, con una cutícula distinguible y contínua. Macropelos ausentes o presentes, cuando presentes unicelulares, de frecuencia variable, de base bulbosa, asociada a células epidérmicas sobreelevadas o en el mismo nivel que el resto de las células epidérmicas. Aguijones no vistos. *Células epidérmicas abaxiales:* células buliformes ausentes. Células epidérmicas grandes y de forma y tamaño regular, con la pared externa notablemente papilosa. Macropelos unicelulares ausentes o presentes, de frecuencia variable. Aguijones no vistos.

Todas las especies de Racemosa se caracterizan por la presencia de células distintivas Kranz en el mesófilo. Las células distintivas Kranz aparecen en transcorte solitarias o en pares y son similares a

las células mestomáticas Kranz que rodean a los haces vasculares. Estas no están acompañadas por tejido vascular y sólo se encuentran asociadas por venillas transversales con los haces vasculares. Carolin et al. (1973) y Sánchez & Arriaga (1988) consideran que la presencia de células distintivas Kranz entre los haces vasculares aseguran un mayor contacto con las células del mesófilo, posibilitando una mayor eficiencia del proceso fotosintético. Hattersley & Watson (1992) sugieren que las células distintivas Kranz representan vestigios o reducciones extremas de haces vasculares de menor orden.

La presencia de este particular tipo de células fue previamente citada para especies de géneros de la tribu Arundinelleae como *Arundinella* (Tateoka, 1956a, 1958; Hattersley & Wattson, 1975; Hattersley et al., 1977; Hattersley & Browning, 1981; Kyoda, 1988; Sánchez & Arriaga, 1988; Sánchez et al., 1989; Elllis, 1990), *Garnottia* (Tateoka, 1956b, 1958; Hattersley & Watson, 1975; Hattersley & Watson, 1992), *Danthoniopsis*, *Loudetia* (Hattersley & Watson, 1992), y *Trichopterix* (Ellis, 1990; Hattersley & Watson, 1992). Estas células también fueron mencionadas en especies de géneros de la tribu Paniceae como *Achlaena* (Hattersley & Watson, 1992), *Anthephora* (Johnson & Brown, 1973), *Arthropogon* (Tateoka, 1963; Hattersley & Watson, 1992), y *Dissochondrus* (Hattersley & Watson, 1992).

Prendergast & Hattersley (1987) citan, dentro del género *Paspalum*, la presencia ocasional de células distintivas Kranz en *P. conjugatum* Berg.; estos autores mencionan en dicha especie la presencia de 3 células distintivas Kranz en el transcorte. Türpe (1966) incluye en su revisión histofoliar de las especies argentinas de *Paspalum* a *P. pygmaeum*, confundiendo a las células distintivas Kranz con pequeños haces vasculares por debajo de las células buliformes.

EPIDERMIS ABAXIAL EN VISTA PARADERMAL

Zonación: zonas costales e intercostales distinguibles; zonas costales angostas, de 2–4 células de ancho; zonas intercostales anchas, de 6–12 células. *Células largas intercostales:* variables en forma, hexagonales a menos frecuente rectangulares en *P. racemosum*, *P. sodiroanum*, *P. pygmaeum* (*Cerrate 1375*), y *P. penicillatum* (*Sneidern 2112*), 3 veces más largas que anchas, con las paredes anticlinales longitudinales paralelas a angulosas, delgadas, paredes anticlinales transversales verticales, paredes periclinales convexas, infladas, más notorias junto a las zonas costales; células largas rectangulares con paredes anticlinales onduladas, las hexagonales usualmente rectas. *Células cortas intercostales:* ausentes. *Aparatos estomáticos:* de 26–32.5 μm de largo, 19.5–23.5 μm de ancho, distribuidos en 3–6 hileras longitudinales en toda la zona intercostal, los contiguos usualmente separados por 1–2 células interestomáticas; células subsidiarias cupuliformes; células largas interestomáticas más de 3 veces más largas que anchas, 2 veces más largas que anchas en *P. pygmaeum* (*Beck 2923*). *Papilas:* ausentes. *Aguijones y ganchos:* ausentes en la zona costal e intercostal, ocasionalmente presentes en *P. candidum*; aguijones de barba corta presentes en el margen de la lámina. *Micropelos:* bicelulares, fusiformes, variables en estructura: de 32.5–39.5 μm de largo, emergiendo la base en ángulo recto o de 52–71 μm de largo, emergiendo la base derecha en *P. candidum*, *P. prostratum*, y *P. sodiroanum*, distribuidos homogéneamente en la zona intercostal; célula basal igual o más corta que la distal, de paredes engrosadas; célula distal de paredes delgadas y ápice ligeramente agudo. *Macropelos:* ausentes o presentes, cuando presentes unicelulares, variables en estructura: de 410–470 μm de largo, de paredes delgadas, asociados a 2 células epidérmicas o de 520–936 μm de largo y de paredes engrosadas, en este caso asociados a células epidérmicas sobreelevadas formando un cojín. *Cuerpos de sílice costales:* de forma variable, halteriformes, de ejes equidimensionales, a longitudinalmente alargados o nodulares, presentes sobre los haces vasculares mayores; *cuerpos de sílice intercostales:* ausentes.

EPIDERMIS ADAXIAL EN VISTA PARADERMAL

Células largas intercostales: similares a las presentes en la cara abaxial. *Células cortas intercostales:* ausentes. *Aparatos estomáticos:* similares a los presentes en la cara abaxial, distribuídos en 2 hileras en la zona intercostal, adyacentes a las zonas costales. *Papilas:* ausentes. *Aguijones y ganchos:* ausentes, ocasionalmente presentes en *P. candidum*. *Micropelos:* como en la cara abaxial. *Macropelos:* de frecuencia variable, de estructura similar a los presentes en la cara abaxial. *Cuerpos de sílice costales:* nodulares a halteriformes, equidimensionales a longitudinalmente alargados; *cuerpos de sílice intercostales:* ausentes.

Discusion

De acuerdo a los caracteres morfológicos se distinguen dentro de Racemosa los siguientes subgrupos de especies:

Subgrupo formado por *P. flavum*, *P. racemosum*, y *P. sodiroanum*, especies que poseen en común espiguillas abiertas en la extremidad a la madurez, con la gluma superior y lemma inferior superando en largo al antecio superior; a su vez ambas brácteas son papilosas en las dos primeras especies y lisas en *P. sodiroanum*. En estas especies el antecio superior es prematuramente caedizo y liso, no papiloso en *P. flavum* y *P. racemosum* y papiloso en *P. sodiroanum*.

Relacionado al subgrupo anterior se encuentran *Paspalum reclinatum* y *P. rugulosum*, teniendo ambas especies espiguillas con antecio más corto que la gluma superior y la lemma inferior. Se distinguen por poseer hojas pseudopecioladas y gluma superior y lemma inferior levemente corrugada en *P. reclinatum* a fuertemente corrugada a lo largo de los márgenes de la gluma superior en *P. rugulosum*. *Paspalum rugulosum* posee, al igual que el subgrupo integrado por *P. candidum* y *P. soukupii*, antecio cartilaginoso.

Otro subgrupo es el integrado por *P. candidum* y *P. soukupii*. Con inflorescencias con espiguillas uniseriadas y racimos terminando en una prolongación estéril; espiguillas con gluma superior ausente, y una posterior reducción con lemma inferior ausente en *P. soukupii*. Además el antecio superior es menos endurecido en *P. candidum* y *P. soukupii* que en las demás especies.

Finalmente *P. penicillatum*, *P. prostratum*, y *P. pygmaeum* se caracterizan por poseer espiguillas con la gluma superior y la lemma inferior tan largas como el antecio superior, con los racimos terminando en una prolongación estéril. Estos caracteres relacionan a estas especies con el subgrupo formado por *P. candidum* y *P. soukupii*; se distinguen de estos taxones por poseer espiguillas con gluma superior presente y antecio superior crustáceo. Dentro de esta agrupación *P. pygmaeum* y *P. penicillatum* se caracterizan por poseer espiguillas biseriadas, raro uniseriadas. *Paspalum prostratum* tiene espiguillas uniseriadas, en ocasiones en 2 series.

Relaciones de Racemosa con Otros Grupos de *Paspalum*

Racemosa se ubica, dentro del género *Paspalum*, junto con otras especies con raquis foliáceo, como las del subgénero *Ceresia* (Pers.) Rchb. y las aquí consideradas en Dissecta y Bonplandiana. El subgénero *Ceresia* incluye especies perennes (con una especie anual, *P. longiaristatum* Davidse & Filg., que es además única dentro del subgénero por poseer gluma superior y lemma inferior largamente aristadas) con raquis manifiestamente foliáceo, el que lleva espiguillas conspicuamente pilosas, cubiertas de largos pelos blanquecinos; las mismas crecen usualmente en lugares abiertos y secos como sabanas, cerrados, campos rupestres, etc. El grupo Dissecta, que incluye aproximadamente unas 8 especies, se distingue de Racemosa por incluir especies perennes, acuáticas o subacuáticas, que habitan en bordes de ríos, lagunas, o en lugares bajos inundables, entre los 0–800 m; las plantas tienen vainas infladas, esponjosas y cañas huecas o cañas con médula esponjosa. Los racimos son persistentes en las inflorescencias, a excepción de *P. longicuspe* y *P. repens*, especies en las que los racimos caen tardiamente. La gluma superior y la lemma inferior son, dentro de las especies de Dissecta, 5-nervias, con 1 par de nervios laterales próximos a los márgenes; en *P. longicuspe* y *P. repens* las brácteas son 2-nervias.

Anatómicamente las especies de Dissecta carecen de células distintivas Kranz.

El grupo Bonplandiana, integrado por 6 especies distribuidas desde Colombia hasta Perú, se halla relacionado a Racemosa por poseer inflorescencias con raquis foliáceo, espiguillas plano-convexas, glabras, con gluma superior y lemma inferior 3-nervias y células distintivas Kranz en el mesófilo foliar. Bonplandiana se distingue de Racemosa por incluir plantas perennes, rizomatosas a largamente estoloníferas, con racimos persistentes en la inflorescencia y por habitar sus especies exclusivamente en páramos entre los 2000 y 4200 m. *Paspalum tuberosum* Mez, especie previamente incluida en el grupo Dissecta por Chase (ined.), y relacionada con las especies anuales de este grupo en un sentido amplio, queda excluida del presente tratamiento, y se la considera dentro de Bonplandiana, por ser una especie perenne, con rizomas cortos, robustos y por tener racimos foliosos, persistentes en la inflorescencia.

Finalmente, y comparando los caracteres presentes en el grupo Racemosa en relación al resto de especies de *Paspalum*, se pueden inferir tendencias evolutivas siguiendo los criterios sustentados por Stebbins (1982), Watson & Dallwitz (1982), y Davidse (1987). Así se podría considerar que los siguientes caracteres son evolucionados en Racemosa: plantas anuales (vs. plantas perennes), raquis de los racimos caedizos (vs. persistentes), raquis foliáceo, ensanchado (vs. raquis filiforme, no ensanchando), y células distintivas Kranz presentes (vs. ausentes). Asimismo dentro del grupo se han observado las siguientes tendencias: láminas pseudopecioladas (vs. láminas no pseudopecioladas), gluma superior ausente (vs. presente), lemma

inferior ausente (vs. presente), y espiguillas uniseriadas (vs. biseriadas).

TRATAMIENTO TAXONOMICO

Paspalum grupo Racemosa

Plantas anuales, con cañas decumbentes, arraigadas y ramificadas en los nudos inferiores, luego erguidas, ocasionalmente plantas arrosetadas. *Lígulas* membranáceas, laciniadas en la porción superior. *Láminas* planas, ovado-lanceoladas, lanceoladas a linear-lanceoladas, de pilosidad variable, excepcionalmente pseudopecioladas. *Inflorescencia* formada por (2–)6–45(–60) racimos ascendentes, caedizos a la madurez; raquis de los racimos foliáceo, ensanchado, glabro, terminando en una espiguilla o en una prolongación estéril; espiguillas solitarias, uni- o biseriadas y dispuestas unilateralmente sobre los racimos; pedicelos cortos, pilosos. *Espiguillas* glabras, ovoides a elipsoides, planoconvexas, pajizas a violáceas. *Gluma inferior* ausente. *Gluma superior* 3-nervia, igualando o superando en largo al antecio superior, ausente en *P. candidum* y *P. soukupii*. *Lemma inferior* glumiforme, 3-nervia, igualando o superando en largo al antecio superior, ausente en *P. soukupii*. *Pálea inferior* ausente; flor inferior ausente; lodículas ausentes. *Antecio superior* cartilaginoso a crustáceo, blanquecino a pajizo o violáceo, liso a papiloso, con cuerpos de sílice o sin los mismos y micropelos bicelulares fusiformes. *Cariopsis* con hilo punctiforme a oblongo, embrión ca. ⅓ del largo de la cariopsis.

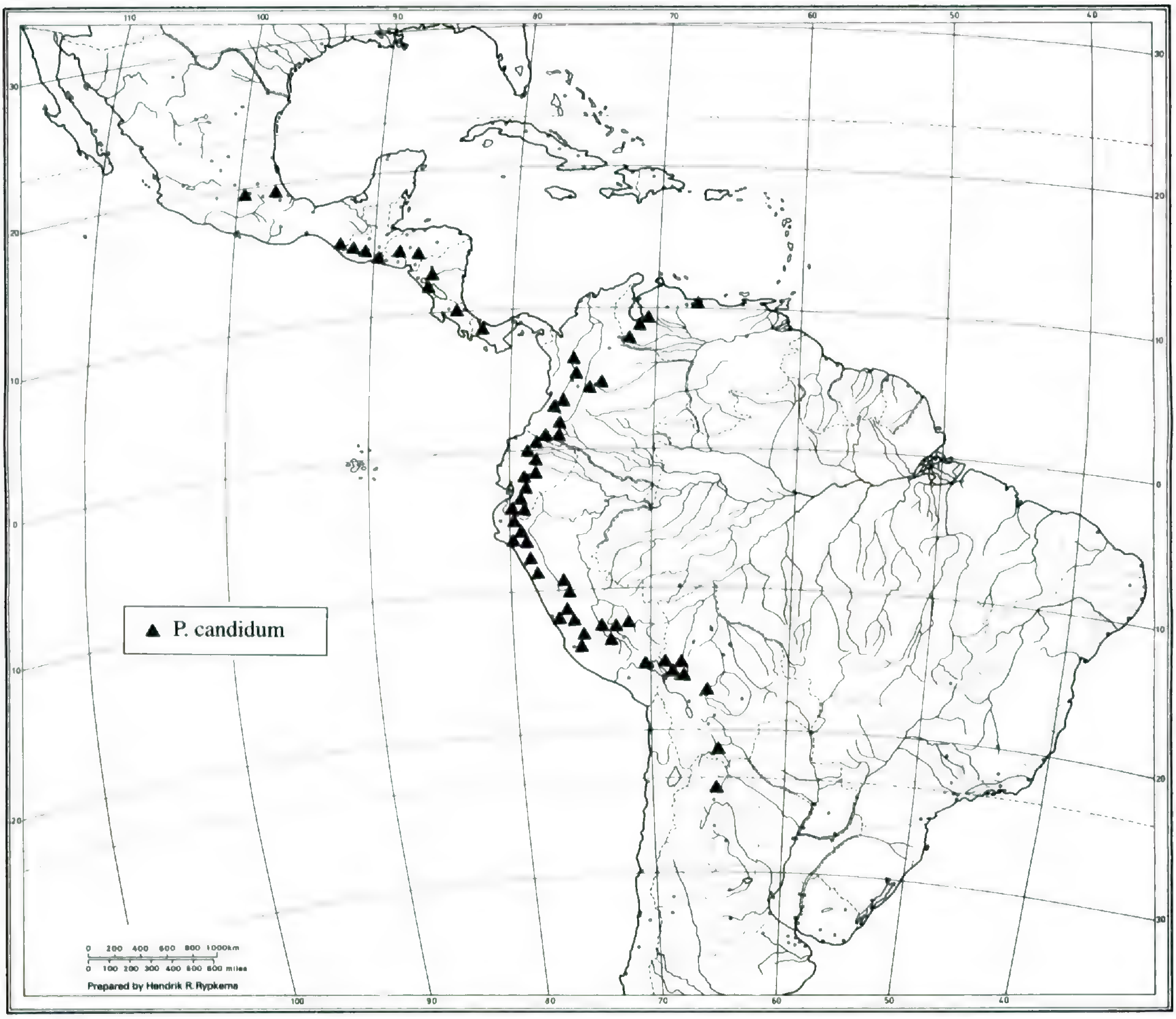
Grupo integrado por diez especies americanas, distribuidas desde México hasta Argentina.

CLAVE PARA LAS ESPECIES DEL GRUPO RACEMOSA

1. Gluma superior ausente 2
1′. Gluma superior presente 3
 2. Lemma inferior ausente ..... 10. *P. soukupii*
 2′. Lemma inferior presente ..... 1. *P. candidum*
  3. Hojas pseudopecioladas 4
  3′. Hojas no pseudopecioladas 5
   4. Antecio superior crustáceo, pajizo a la madurez; gluma superior lisa a levemente corrugada en la porción inferior; raquis de 1–2 mm de ancho ..... 7. *P. reclinatum*
   4′. Antecio superior cartilaginoso con tintes violáceos a completamente violáceo a la madurez; gluma superior fuertemente corrugada a lo largo de los márgenes; raquis de 0.5–0.8 mm de ancho ..... 8. *P. rugulosum*
    5. Antecio superior caedizo, 0.5–1.5 mm más corto que la gluma superior y lemma inferior; espiguillas abiertas a la madurez 6
    5′. Antecio superior persistente, tan largo como la gluma superior y lemma inferior; espiguillas cerradas a la madurez 8
     6. Gluma superior y lemma inferior lisas, no papilosas; antecio superior 1.8–2.4 mm de largo, papiloso; racimos rectos ..... 9. *P. sodiroanum*
     6′. Gluma superior y lemma inferior lisas a transverso-rugosas, papilosas; antecio superior de 1–1.7 mm de largo, liso, racimos rectos o arqueados 7
      7. Gluma superior y lemma inferior transverso-rugosas, con papilas infladas en la porción inferior; ápice de la gluma y lemma inferior ciliados ..... 6. *P. racemosum*
      7′. Gluma superior y lemma inferior sin arrugas transversales, finamente papilosas, glabras ..... 2. *P. flavum*
       8. Lemma inferior de dorso profundamente surcado en la porción media ..... 3. *P. penicillatum*
       8′. Lemma inferior de dorso plano a suavemente cóncavo, no profundamente surcada en la porción media 9
        9. Espiguillas uniseriadas de (1.9–)2.1–2.7 mm de largo; plantas de 20–50 cm de alto; láminas lanceoladas de 3–10 cm de largo, 0.3–1 cm de ancho ..... 4. *P. prostratum*
        9′. Espiguillas biseriadas de 1.5–2.1 mm de largo; plantas de 1–8(–15) cm de alto; láminas linear-lanceoladas de 1–5.5 cm de largo, 0.15–0.4 cm de ancho ..... 5. *P. pygmaeum*

1. Paspalum candidum (Humb. & Bonpl. ex Flüggé) Kunth, Mém. Mus. Hist. Nat. 2: 68. 1815. *Reimaria candida* Humb. & Bonpl. ex Flüggé, Gram. Monogr., Paspalum.: 214. 1810. TIPO: Ecuador. Pichincha: Puanbo [error por Puembo], *Humboldt & Bonpland s.n.* (holótipo, B-W no visto, fragmento, US-2942124; isótipo, P, fragmento, US-2942124). Figuras 4–6.

Paspalum depauperatum J. Presl, Reliq. Haenk. 1: 215. 1830. TIPO: Perú: "habitat in Peruviae montanis huanoccensibus," *Haenke s.n.* (holótipo, PR no visto, fragmento y foto, US-2942406).



Paspalum confertum Willd. ex Steud., Nomencl. Bot. (ed. 2) 2: 270: 1841, pro syn., non LeConte, 1820.
Paspalum uniseriatum Steud. ex Lechler, Berberid. Amer. Austral.: 55. 1857, nom. nud.
Paspalum scabrum Scribn., U.S.D.A. Div. Agrostol. Bull. 4: 36, pl. 3. 1897. TIPO: Guatemala. Santa Rosa: Chupadero, 5000 ft., 1892, *Heyde & Lux 3903* (holótipo, US-741453; isótipos, US-207233, 824321).
Paspalum lineispatha Mez, Repert. Spec. Nov. Regni Veg. 15: 27. 1917. TIPO: Perú. Ancash: Hacienda Cajabamba, entre Samanco y Casaz [error por Caraz], 3000–3500 m, Mayo, *Weberbauer 3142* (holótipo, B no visto, fragmento, US-2855794).
Paspalum vinosum Mez, Repert. Spec. Nov. Regni Veg. 15: 28. 1917. TIPO: Bolivia. La Paz: Yungas austr., Sirupaya prope Yanacachi, *Buchtien 1408* (lectótipo, aquí designado, B; isolectótipo, M).
Paspalum sincoranum Mez, Bot. Jahrb. Syst. 56 (Beibl. 125): 9. 1921. TIPO: Brasil. Bahia: sin localidad, *De Brixen s.n.* (holótipo, B no visto, fragmento, US-2856023).

Plantas anuales, decumbentes, arraigadas y ramificadas en los nudos inferiores, luego ascendentes, apoyantes o erectas, con pocas a numerosas ramificaciones en los nudos inferiores; *cañas* de 8–120 cm de alto; entrenudos de 2–23 cm de largo, glabros, comprimidos, pajizos, violáceos o con tintes purpúreos; nudos oscuros, esparcidamente pilosos a glabros. *Vainas* de 1.5–16 cm de largo, las inferiores hirsutas, con pelos papilosos, blanquecinos, caedizos a glabras, lisas o escabrosas, los márgenes cortamente ciliados en toda su superficie o sólo hacia la porción superior, luego glabros. *Lígulas* de 0.5–3 mm de largo, hialinas, castañas, la cara abaxial cortamente pilosa, con pelos blanquecinos. *Láminas* ovado-lanceoladas a lanceoladas, de 1.5–9(–14) cm de largo, 0.3–1.2(–3) cm de ancho, herbáceas, de base redondeada, no pseudopecioladas, y ápice agudo, esparcidamente papiloso-pilosas en ambas superficies, más hacia la región basal, a hirsutas, los márgenes escabrosos. *Pedúnculos* hasta de 30 cm de largo, angulosos, glabros, lisos a escabrosos hacia la porción superior. *Inflorescencias* exertas, de contorno

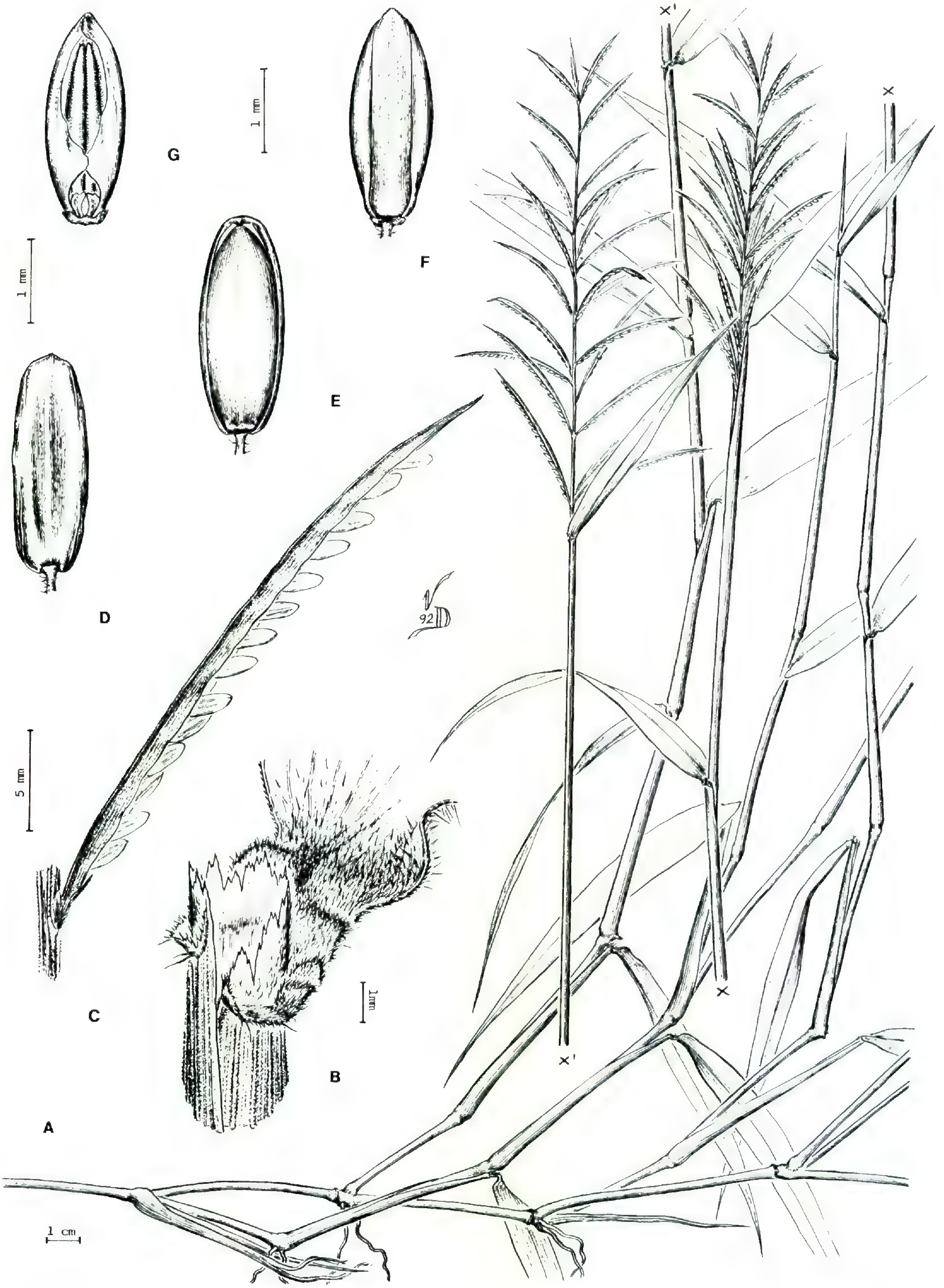


FIGURA 5. *Paspalum candidum* (Humb. & Bonpl. ex Flüggé) Kunth (de *Wood 4357*).—A. Hábito.—B. Detalle de región ligular.—C. Racimo de la inflorescencia.—D. Espiguilla vista del lado de la lemma inferior.—E. Espiguilla vista del lado de la lemma superior.—F. Antecio superior visto del lado de la pálea.—G. Pálea superior con lodículas y estambres.

FIGURA 6. Ejemplar depauperado de *Paspalum candidum* Humb. & Bonpl. ex Flüggé (de *Wurdack 572*). —A. Hábito. —B. Detalle de la región ligular. —C. Racimo de la inflorescencia. —D. Espiguilla vista del lado de la lemma inferior. —E. Espiguilla vista del lado de la lemma superior. —F. Antecio superior visto del lado de la pálea. —G. Pálea superior con lodículas y estambres.

elíptico, de 4–30 cm de largo, 1.5–6(–9) cm de ancho; eje principal anguloso, glabro, liso a escabroso, en ocasiones terminando en una prolongación estéril; pulvínulos pilosos, con pelos blanquecinos, delicados; ramificaciones (3–)6–45, alternas, ocasionalmente subopuestas, divergentes; raquis de las ramificaciones de 0.5–4 cm de largo, 1–2.8 mm de ancho, terminando en una prolongación estéril, verdoso a violáceo, la cara abaxial cortamente pilosa, finamente escabriúscula, con quilla conspicua, cara adaxial glabra, escabriúscula a lisa, los márgenes escabrosos; pedicelos de 0.2–0.3 mm de largo. *Espiguillas* elipsoides de (1.3–)1.5–3(–3.3) mm de largo, 0.7–1 mm de ancho, uniseriadas, pálidas o con tintes purpúreos. *Gluma superior* ausente, en ocasiones reducida a un rudimento. *Lemma inferior* tan larga como la espiguilla, membranácea, delicada, lisa, glabra, cubriendo el ápice del antecio superior a un poco más estrecha, 3-nervia. *Antecio superior* elipsoide, de (1.2–)1.4–3 mm de largo, 0.6–0.9 mm de ancho, glabro, cartilaginoso, blanco-sedoso o con tintes purpúreos, brillante, finamente papiloso; lemma totalmente expuesta, 3-nervia; con papilas simples en toda la superficie y cuerpos de sílice exfoliados hacia el ápice; pálea 2-nervia, con papilas simples en toda la superficie, micropelos bicelulares presentes hacia los bordes junto a la lemma y cuerpos de sílice exfoliados hacia el ápice; lodículas 2, de 0.2 mm de largo, abrazando los bordes inferiores de la pálea; estambres 3, anteras de 0.8–1.9 mm de largo, purpúreas. *Cariopsis* elipsoide, de 1.2–1.6 mm de largo, 0.6 mm de ancho; hilo oblongo; embrión ⅓ del largo de la cariopsis.

Nombre vulgar. "Nudillo" (Colombia); "terrecillo," "zacate de agua" (Costa Rica); "fuquillo," "nudillo" (Ecuador); "uluzacate" (El Salvador); "matash," "gengibrillo," "grama de agua" (Guatemala); "nudillo," "manga larga" (Perú).

Distribución y ecología. Habita desde México hasta el NW de Argentina y en Chile en Valparaíso. Crece en zonas montañosas, en el sotobosque de selvas nubladas y bosques o en bordes de arroyos, en ambientes húmedos, entre los (300–)1000–4600 m. Es una gramínea forrajera, especial para el ganado (de acuerdo a la etiqueta del ejemplar *Acosta Solís 18487* y *Asplund 8220*). Ocasionalmente presente como maleza en cultivos.

Material adicional examinado. ARGENTINA. **Jujuy:** Capital, Lagunas de Yala, 2000 m, 26 Mar. 1979, *Cabrera et al. 30677* (US), 23 Mar. 1993, *Deginani & Cialdella 101** (SI); Abra de Lozano, 24 Mar. 1993, *Deginani & Cialdella 135** (SI). BOLIVIA. **Cochabamba:** Comarapa to Cochabamba, 2650 m, 24 Mar. 1981, *Renvoize & Cope 4069* (MO, US). **La Paz:** Sud Yungas, Sirupaya bei Yanacachí, 2100 m, 13 Nov. 1906, *Buchtien s.n.* (US 1099146), *422* (LIL); vicinity of Sorata, 2650–2800 m, *Mandon 1251*** (US); Murillo, Valle de Zongo, desvío por el pueblo de Zongo, 2200 m, 29 Abr. 1981, *Beck 4687** (LPB). **Tarija:** Méndez, Cuesta de Sama, 2500 m, 28 Feb. 1960, *Meyer et al. 21430* (LIL). CHILE. **Valparaiso:** near Valparaíso, 1926, *Gunther 33* (US). COLOMBIA. **Antioquia:** abajo de Bocaná a 7 km al E de Medellín, 1800 m, 20 Feb. 1949, *Araque Molina & Barkley 19An180* (US). **Caldas:** Jardín, a las orillas de la carretera entre Jardín y Río Sucio, 12 km de Jardín, 05°32'N, 79°49'W, 2340 m, 28 Ene. 1989, *McDougal & Roldán 3489* (MO, SI). **Cauca:** El Tambo, 1700 m, 25 Jun. 1938, *von Sneidern 1287* (US). **Cundinamarca:** between Anolaima and Cachipay, 17 Abr. 1935, *Archer 3327*** (US). **Huila:** El Estrecho near San Agustín, 1300 m, 20 Abr. 1984, *Wood 4357* (COL). **Nariño:** vía Túquerres–Yumaco, Río Laverde, El Guabo, 2200 m, 7 Mar. 1987, *Londoño & Quintero 229* (MO). **Norte de Santander:** headwaters of Quebrada Bagueche, 5700 ft., 10 Abr. 1944, *Fassett 25046* (US). **Putumayo:** Valle de Sibundoy, 5 km S of Sibundoy, 2200 m, 12 Jul. 1963, *Bristol 1202** (US). **Santander:** vicinity of California, 2000 m, 11/27 Ene. 1927, *Killip & Smith 16969, 17018* (US). **Tolima:** Ravine, 21/25 Dic. 1917, 1100–1300 m, *Pennell 3318* (US). COSTA RICA. **Alajuela:** Viento fresco, NE of San Isidro, 1600–1900 m, 13 Feb. 1926, *Standley & Torres 47838* (US). **Cartago:** Río Reventado, Abr. 1894, *Smith 4992* (US). **Heredia:** San Rafael, 1100 m, 2 Ene. 1950, *León 2059* (US). **San José:** Cordillera de Talamanca, 14 km S of División along the Interamericana Highway, 1500 m, 13 Dec. 1968, *Pohl & Davidse 11617* (MO, US). ECUADOR. **Azuay:** 3–8 km N of Sevilla de Oro, 7000–8300 ft., 3 Sep. 1945, *Camp E-5217* (MO, US). **Bolívar:** 9 km NE of Balzapamba on road to San Miguel, 1770 m, 5 Mayo 1990, *Peterson & Annable 9025* (MO). **Carchi:** El Chamizo, 2990 m, 23 Jun. 1950, *Acosta Solís 16634* (US). **Chimborazo:** vicinity of Huigra, mostly on the Hda. de Licay, 2 Sep. 1918, *Rose & Rose 22582** (US); Alausí, 2500 m, 19 Jul. 1923, *Hitchcock 20723*,*** (US). **El Oro:** Puyango and vicinity, 300–900 m, Ago. 1978, *Daly 131* (MO). **Imbabura:** San Francisco de Sigsipamba, lado interior de la Cordillera Oriental, 2500 m, 30 Jul. 1950, *Acosta Solís 18487** (US). **Loja:** 1 km W of Purinuma on road to Gonzanamá, 2430 m, 2 Jun. 1990, *Peterson & Judziewicz 9464* (MO, US). **Pichincha:** Pululagua, 2500 m, 6 Jun. 1939, *Asplund 6715* (MO). **Tungurahua:** Píllaro, 2850 m, 10 Ago. 1939, *Asplund 8147, 8148* (US). EL SALVADOR. **Ahuachapán:** vicinity of Ahuachapán, 1000 m, 9/27 Ene. 1922, *Standley 19787* (US). **San Salvador:** Ataco, 1928, *Calderón 2434* (US). **Sin Departamento:** Volcans of El Salvador, 600–700 m, 20/26 Nov. 1911, *Hitchcock 8937* (SI, US). GUATEMALA. **Alta Verapaz:** Cobán, 1350 m, Nov. 1912, *von Türckheim 3970* (LIL). **Guatemala:** between Antigua and Guatemala City, ⅓ Dic. 1911, *Hitchcock 9144* (US). **Jalapa:** mountains about Chahuite, NW of Jalapa, 1650 m, 16 Nov. 1940, *Standley 77431* (US). **Sacatepéquez:** Cuesta de las Cañas, above Antigua, 6 Dic. 1938, *Standley 58967* (US). **San Marcos:** between San Rafael Pie de la Cuesta and Palo Gordo, 1800–2400 m, 10/18 Dic. 1963, *Williams et al. 25967* (US). **Sololá:** Sololá, 31 Ene. 1915, *Holway 168* (US). **Santa Rosa:**

Laguna de Carrizal, Ene. 1893, *Heyde & Lux 4300* (US). **Quezaltenango:** Almolonga, 2150 m, Ago. 1954, *Koninck 76* (US). HONDURAS. **Intibucá:** above Yamaranguita, Cerro Yamaranguila, 2000 m, 27 Nov. 1958, *Hawkes 2090* (US). **Francisco Morazán:** La Montañita, 1650 m, 29 Dic. 1947, *Williams & Molina 13613* (US). MEXICO. **Chiapas:** Escuintla, 6 Mayo 1936, *Matuda 318* (US). **Morelos:** Teposteco, Dic. 1936, *Lyonnet 1371* (US). **Veracruz:** Barraca of Texolo, near Jalapa, 3500 ft., 30 Abr. 1899, *Pringle 7884* (US). NICARAGUA. **Managua:** vicinity of Managua, *Garnier 446* (US). **Matagalpa:** Santa María de Ostuma, Cordillera Central de Nicaragua between Matagalpa and Jinotega, 1300–1500 m, 8/15 Ene. 1963, *Williams et al. 23404* (US). PANAMA. **Chiriquí:** steep rocky face and trailside along the Río Caldera beyond Bajo Mono ca. 4 mi. NW of Boquete, 4200 ft., 22 Ene. 1971, *Wilbur et al. 13525* (MO, US). PERU. **Amazonas:** Chachapoyas, rocky slopes of Caño Santa Lucía, 2000–2400 m, 28 Mayo 1962, *Wurdack 572* (US). **Ancash:** Huaráz, 10 km by road from Cachabamba, 77°51′W, 9°27′S, 2870 m, 6/8 Jun. 1985, *Smith & Buddensiek 10901* (MO, SI, US). **Apurímac:** Abancay, Ompay, 3600 m, Jun. 1938, *Vargas 1005* (LIL). **Ayacucho:** Pampalca, between Huanta and Río Apurimac, 3200 m, 4/5 Mayo 1929, *Killip & Smith 22242**,** (US). **Cajamarca:** Celendín-Balsas, 2850 m, *Sagástegui et al. 12196* (MO, US); San Miguel, Cerro Quillón, Agua Blanca, 3100 m, 5 Jul. 1986, *Mostacero et al. 1248* (MO, SI). **Cuzco:** Urubamba, Machu Picchu, circuito de Camino Inca, camino de Huiñay-huaina a Intipunco, 13°09′S, 72°31′W, 2000–4200 m, Oct. 1986, *Tupayachi 228* (MO, SI). **Huancavelica:** Tayacaja, Chuspi Hacienda, Tocas, entre Colcabamba y Paucarbamba, 2900 m, 22 Abr. 1954, *Tovar 2040** (US, USM). **Huánuco:** road from Huánuco to Tingo María, N of Carpish Pass, 48.6 km NE of Huánuco, 2490 m, 6 Dic. 1981, *Plowman & Rury 11128* (MO). **Ica:** San Juan, 2600 m, 17 Jun. 1965, *Sánchez 76* (US). **Junín:** Huacapistana, 1800–2400 m, 5/8 Jun. 1929, *Killip & Smith 24129**,** (US). **La Libertad:** Otuzco, Cerro Ragache, Salpo, 3200 m, 23 Mayo 1984, *Sagástegui et al. 11592*** (MO, US). **Lambayeque:** Lambayeque, entre Huratara y Colaya, 2000 m, 5 Jul. 1986, *Llatas Quiróz 1983* (MO). **Lima:** Omancaes, 300 m, 8 Ago. 1945, *Infantes 1652* (LIL). **Pasco:** Oxapampa vía Río Yamaquizu, 75°17′W, 10°30′S, 1870 m, 2 Jun. 1982, *Smith et al. 1762* (MO, SI). **Piura:** Huancabamba, La Beatita, 1200 m, 20 Abr. 1986, *Llatas Quiróz 1872* (MO, SI). **Puno:** Sandía, abajo de Cuyo-Cuyo, 3000 m, 13 Mayo 1966, *Ferreyra 16635* (USM). **Tumbes:** Zarumilla, Bosque Nac. de Tumbes, 600–800 m, *Simpson & Schunke 463* (US). VENEZUELA. **Aragua:** prope Colonia Tovar, 1856–1857, *Fendler 1695* (MO, US). **Distrito Federal:** Caracas, Silla de Caracas, 1891–1892, *Warmings s.n.* (US 1258782). **Lara:** Jiménez, El Blanquito, Parque Nacional Yacambú, 15 Feb. 1978, *Burandt Jr. V0057a* (MO). **Mérida:** 7 mi. SE of Santo Domingo along Mérida–Barinas Hwy., 1700 m, 19 Nov. 1971, *Davidse 3199* (MO, US). **Táchira:** arriba de Cementos Táchira, hacia Borotá, 1400–1500 m, 28 Feb. 1985, *Bono 4637* (MO).

Chase (1927, 1929), Hitchcock (1930), Swallen (1955), y más recientemente Pohl (1980) y Tovar (1993) trataron separadamente a *P. candidum* y *P. scabrum*, distinguiendo a la primera especie por tener vainas lisas a ligeramente escabrosas y espiguillas mayores de 2 mm de largo, y a *P. scabrum* por ser plantas escabrosas con espiguillas menores de 2 mm de largo. Al revisar abundante material se observó una marcada variación en los caracteres previamente mencionados. Así, se hallaron ejemplares con vainas escabrosas y espiguillas de 2.3–2.7 mm de largo, como por ejemplo *Asplund 7440, 8220*; *Ariste-Joseph A550, Maurice 657, Burandt Jr. V0057a, Fassett 25046, Peterson et al. 8831, Pittier 1238* y *Wood 4357*, o ejemplares con vainas lisas y espiguillas de 1.3–1.8 mm de largo (*Acosta Solís 11367, Sagástegui et al. 9322*). La textura de las vainas es variable en un mismo ejemplar, hallándose vainas lisas y otras conspicuamente escabrosas en un mismo espécimen, como en *Hutchinson & Wright 3468, Infantes 1652, Killip & Smith 17018, LLatas Quiróz 1872, Reed 199, Peterson & Annable 9025, Peterson & Judziewicz 9464, Rimbach 766*, y *Wood 4357*. Consecuentemente, en el presente tratamiento se considera que la textura de las vainas y el tamaño de la espiguilla no son caracteres suficientemente consistentes para la segregación de ambos taxones, incluyéndose a *P. scabrum* en la sinonimia de *P. candidum*.

Asimismo Chase (1927) y Tovar (1993) distinguen a *P. depauperatum* y *P. lineispatha* de *P. candidum* por incluir plantas menores de 80 cm de alto y espiguillas de menos de 2 mm de largo. El análisis de material de herbario permitió confirmar que existe una total gradación en los caracteres previamente mencionados, considerándose a ambos taxones en la sinonímia de *P. candidum*.

En la descripción original de *P. depauperatum* J. Presl señala que posee gluma inferior brevísima e inflorescencias con 4–5 racimos. No se observó gluma inferior en el ejemplar tipo examinado de la especie y el número bajo de racimos descriptos seguramente se deba a que los mismos son deciduos.

Se ha designado como lectótipo de *P. vinosum* al ejemplar *Buchtien 1408* del herbario de Berlín (B) por tratarse del espécimen estudiado por Mez al describir la especie, incluyendo este ejemplar los datos de la localidad tipo de la especie. El ejemplar *Buchtien 422* posee los mismos datos de la localidad y fecha por lo que es posible que exista un error de etiquetado con este último espécimen. Mez describió erróneamente a esta especie como perenne pues el ejemplar tipo es una planta anual, decumbente y arraigada en los nudos inferiores. En US hay un fragmento 80040 (del herbario de Berlín), *Buchtien s.n.*, Sirupaya pr. Yanacachi, 13 Nov. 1906, 2100 m, Bolivia, y un ejemplar US

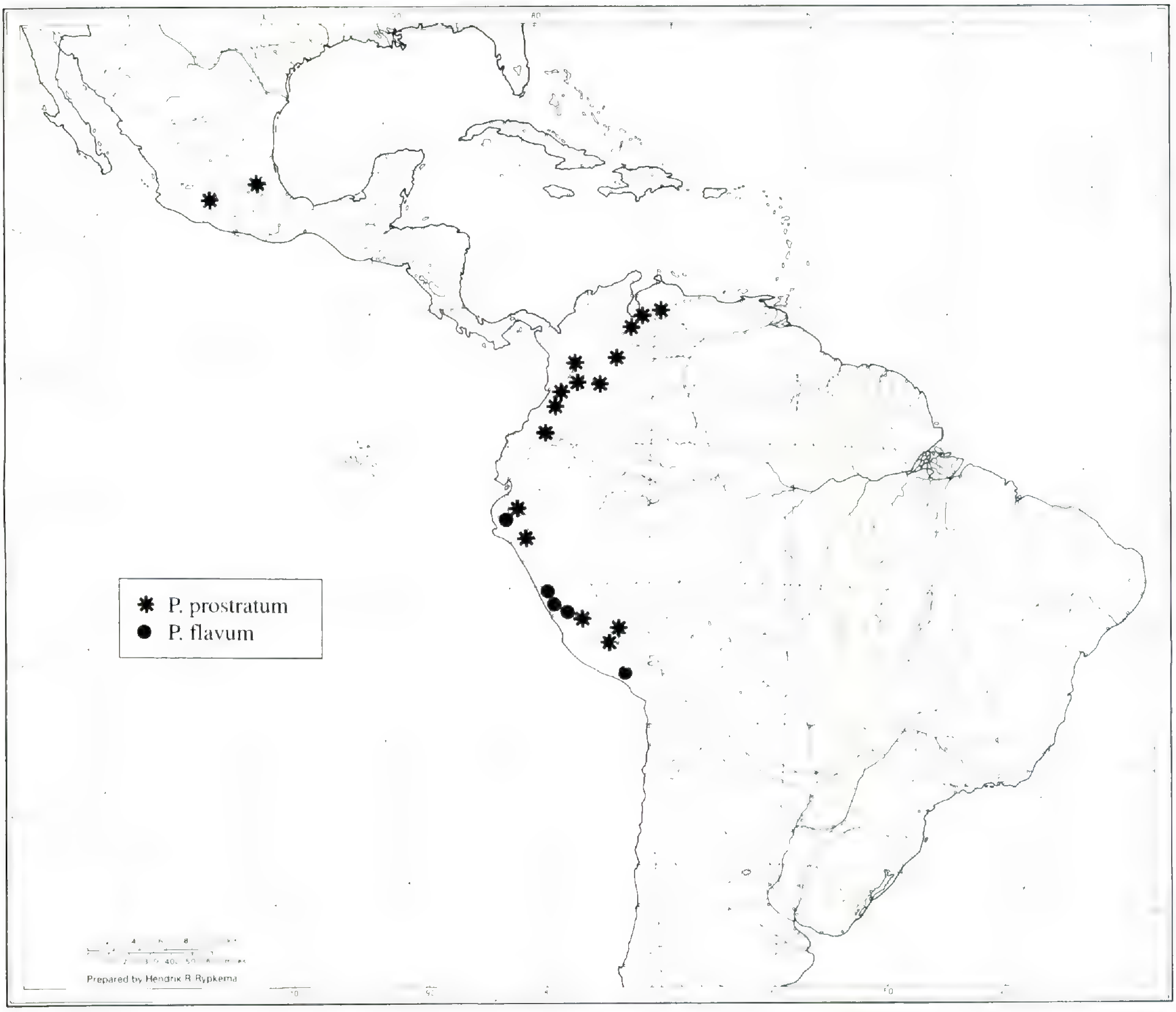


FIGURA 7. Distribución de *P. flavum* y *P. prostratum.*

1099146 con los mismos datos que el anterior *Buchtien 422.*

Al describir *P. sincoranum,* Mez se basó en un material colectado por De Brixen aparentemente en la Serra do Sincorá en el sur de Bahia, Brasil. Sin embargo, hasta la actualidad no se ha registrado su presencia en el Brasil y probablemente exista en la mencionada cita un error en la localidad de colección (Chase, ined.).

Paspalum candidum se caracteriza por tener espiguillas sin gluma superior y distribuidas en 1-serie a lo largo del raquis de los racimos. Cabe destacar que ocasionalmente se ha observado espiguillas aisladas en la inflorescencia con una gluma superior rudimentaria, como por ejemplo en *Fassett 25046, Reed 199,* y *Rose & Rose 22582.* También se observaron ocasionalmente espiguillas dispuestas en 2-series a lo largo de los racimos, hacia la base de los mismos, en los especímenes *Bristol 1202, Garnier 446, 4528, Davidse & D'Arcy 10149, Hammel 1397,* y *Williams 17305.*

Normalmente esta especie se encuentra a elevaciones entre 1000 y 4600 m; por debajo de los 1000 m se examinaron ejemplares aislados de Chile, Perú, Ecuador, y El Salvador.

2. Paspalum flavum J. Presl, Reliq. Haenk. 1: 220. 1830. TIPO: Chile. Sin localidad, *Bertero s.n.* (holótipo, PR no visto, fragmento y foto, US-2855336). Figura 7.

Paspalum cristatum Trin., Mem. Acad. Imp. Sci. St.-Pétersbourg Sér. 6, Sci. Math. 1: 152. 1834. TIPO: Chile, "V. spp. Chil.", *Cuming 1054.* (holótipo, LE no visto; isótipo, BRU no visto, fragmento, US-929181).

Paspalum mollendense Mez, Repert. Spec. Nov. Regni Veg. 15: 31. 1917. TIPO: Perú. Mollendo, 50–100 m, *Weberbauer 1553* (holótipo, B no visto, fragmento, US-2855818).

Plantas anuales, de 15–90 cm de alto, con *cañas* geniculadas a decumbentes, ascendentes, ramificadas y arraigadas en los nudos inferiores a

erectas; entrenudos de 4–20 cm de largo, hasta de 4 mm de ancho, cilíndricos, huecos, amarillos a rojizos; nudos glabros o en ocasiones pilosos, castaños. *Vainas* de 3.5–10 cm de largo, glabras a pilosas junto a la porción superior, con pelos delgados y largos, los márgenes membranáceos. *Lígulas* de 1–2 mm de largo, hialinas, castañas. *Láminas* ovado-lanceoladas a lanceoladas, de 1.9–17 cm de largo, 4–15 mm de ancho, herbáceas, delgadas, glabras a esparcidamente pilosas, de base cordada a subcordada, no pseudopecioladas, y ápice agudo, los márgenes ciliados a escabrosos. *Pedúnculos* hasta de 30 cm de largo, angulosos. *Inflorescencias* angostamente elipsoides, densifloras, de 3–15 cm de largo, 3–4 cm de ancho; eje principal anguloso, surcado, glabro o con pelos cortos hacia el ápice, liso a escabriúsculo; pulvínulos pilosos; ramificaciones 15–60, alternas a subopuestas, ascendentes, divergentes, arqueadas; raquis de los racimos de 0.5–3.5 cm de largo, 0.5–1 mm de ancho, terminando en una espiguilla, verdoso, delgado, aquillado, con quilla pilosa, glabro a cortamente pubescente en ambas caras, en ocasiones escabroso; pedicelos de 0.4–0.8 mm de largo, aplanados, delgados. *Espiguillas* ovoides a ovoideo-elipsoides, de 1.5–2 mm de largo, 0.7–0.9 mm de ancho, abiertas a la madurez, biseriadas, imbricadas, glabras, ferrugíneas, purpúras o amarillas. *Gluma superior* y *lemma inferior* tan largas como la espiguilla, membranáceas, finamente papilosas, glabras, lisas, subiguales, superando en largo el antecio superior, abiertas hacia el ápice y con los nervios tenues. *Antecio superior* elipsoide, de 1–1.7 mm de largo, 0.5–0.8 mm de ancho, crustáceo, blanco sedoso a castaño, liso, brillante, no papiloso, caedizo a la madurez, 0.5–0.8 mm más corto que la espiguilla; lemma y pálea con cuerpos de sílice exfoliados hacia el ápice; estambres 3, anteras de 1.2–1.4 mm de largo; lodículas 2, de 0.2 mm de largo, abrazando los bordes inferiores de la pálea; estilos libres. *Cariopsis* elipsoide, de 1.2 mm de largo, 0.6 mm de ancho, pajizo; hilo punctiforme, embrión ⅓ o un poco más del largo de la cariopsis.

Nombre vulgar. "Gaiga" (Perú).

Distribución y ecología. Habita en Chile y Perú, en faldas pedregosas de cerros, entre 50 y 3500 m.

Material adicional examinado. PERU. **Ancash:** Bolognesi, Timpoc, abajo de Chiquián, 2600–3500 m, 12 Mayo 1950, *Ferreyra 7357* (US). **Junín:** Huancayo, 3500 m, 8 Ago. 1945, *Infantes 894* (LIL). **Lima:** above San Mateo, 3700 m, 22 May 1948, *Anderson 699* (US); along Río Chillón, near Viscas, 1800–2000 m, 10–15 Jun. 1925, *Pennell 14461* (US); Morro Solar, near Chorillos, 250 m, Sep. 1910, *Weberbauer 5707*** (US); Matucana, 8000 ft., 12 Abr. 3 Mayo 1922, *MacBride & Featherstone 135* (US); Yauyos, Cunuco, abajo de Tupe, 2300 m, 8 Ene. 1952, *Cerrate & Tovar 1124** (US); Huarochirí, Surco, 2000 m, 24 Mayo 1940, *Asplund 11062** (US); vicinity of Lima, San Agustín, 27 Sep. 1940, *Asplund 13787** (US); Huarochiri, S of Matucana along carretera Central, 11°50′S, 76°20′W, 2000–3000 m, 9 Mayo 1981, *Sullivan et al. 844* (MO).

Las únicas colecciones de Chile de esta especie son las que corresponden a los tipos de *P. flavum* y *P. cristatum*.

3. Paspalum penicillatum Hook. f., Trans. Linn. Soc. London 20: 171. 1847. TIPO: Ecuador. Archipiélago de Colón: Charles Island, 1835, *Darwin s.n.* (lectótipo, designado por Porter, 1980, CGE no visto; isolectótipo, K no visto, fragmento, US-2942530). Figura 8.

Plantas anuales, con *cañas* decumbentes a geniculadas, arraigadas y ramificadas en los nudos inferiores, luego suberectas, ascendentes, de (10–)20–100(–200) cm de largo; entrenudos de 1.5–16 cm de largo, huecos, cilíndricos, verdosos a violáceos, brillosos; nudos glabros a esparcidamente pilosos, castaños. *Vainas* de 1.5–7 cm de largo, usualmente más cortas que los entrenudos, híspidas, con pelos papilosos hacia la región superior a cortamente pilosas en el resto de la superficie, los márgenes ciliados. *Lígulas* de 1–2.5 mm de largo, hialinas, largamente pilosas en la cara abaxial. *Láminas* ovado-lanceoladas a lanceoladas, de 1.5–12 cm de largo, 0.4–1.5 cm de ancho, herbáceas, de base redondeada, no pseudopecioladas, y ápice agudo, hirsutas a esparcidamente pilosas en la cara adaxial o sobre ambas caras, los márgenes escabriúsculos, en ocasiones ciliados, nervio medio prominente en la cara abaxial. *Pedúnculos* delgados, de 2.5–15 cm de largo, angulosos, glabros. *Inflorescencias* exertas a subincluídas, de 3–15 cm de largo, 1–8 cm de ancho; eje principal anguloso a aplanado, escabroso, terminando en un racimo desarrollado o reducido en una prolongación estéril; pulvínulos densamente pilosos, con pelos blanquecinos hasta de 2.8 mm de largo; ramificaciones 5–20, ascendentes, alternas, subopuestas a subfasciculadas; raquis de las ramificaciones de 1–4.5 cm de largo, 1–2.4 mm de ancho, verdoso, terminando en una prolongación estéril, con la cara adaxial pubescente o glabra y la cara abaxial cortamente pubescente, los márgenes escabriúsculos, el nervio medio blanquecino, flexuoso, escabroso; pulvínulos densamente pilosos, con pelos blanquecinos hasta de 2.8 mm de largo; pedicelos aplanados, de 0.5 mm de

FIGURA 8. Distribución de *P. penicillatum*.

largo. *Espiguillas* elipsoides, de 1.2–1.9 mm de largo, 0.6–0.8 mm de ancho, cerradas a la madurez, solitarias, biseriadas, ocasionalmente uniseriadas, verde-blanquecinas, rara vez con tintes purpúreos, de ápice agudo. *Gluma superior* y *lemma inferior* tan largas como el antecio superior, glabras, lisas, 3-nervias, los nervios manifiestos; lemma inferior longitudinalmente surcada en la región central. *Antecio superior* elipsoide, de 1.1–1.8 mm de largo, 0.5–0.8 mm de ancho, persistente, glabro, crustáceo, pálido, brillante; lemma aguda, lisa, papilosa, con cuerpos de sílice exfoliados hacia el ápice; pálea papilosa con papilas simples, tenues; estambres 3, anteras de 0.5 mm de largo; lodículas 2, de 0.3 mm de largo. *Cariopsis* elipsoide, de 1.2 mm de largo, 0.6 mm de ancho; hilo punctiforme; embrión ⅓ del largo de la cariopsis.

Nombre vulgar. "Ilín," "saraquigua" (Ecuador); "nudillo" (Perú).

Distribución y ecología. Crece en Venezuela, Colombia, Ecuador, Perú, y Bolivia, en terrenos cultivados o modificados, en laderas húmedas en zonas montañosas, entre 150 y 4000 m.

Material adicional examinado. BOLIVIA. **Cochabamba:** Punata, comunidad Lara Suyo, 2700 m, 26 Abr. 1991, *Guillén 471* (LPB); Cochabamba, 2566 m, *Spiaggi 23* (US). **La Paz:** Sur Yungas, Sirupaya, 10 Dic. 1906, *Buchtien 417* (US); Sur Yungas, Villa Aspiazu, Jun. 1920, *Holway & Holway 697* (BAA, US); Sud Yungas, 16 km E of (below) Chuspipata on the road to Chulumani, 16°20′S, 67°49′W, 2100 m, 29 Sep. 1985, *Solomon & Nee 14340* (MO, SI, US); Nor Yungas, 0.7 km W of Chuspipata, 16°18′S, 67°49′W, 3100 m, *Solomon 13692* (MO); Sorata, Mayo 1892, *Bang 1310* (MO, US), *Gunther 68** (US); Nord Yungas, región von Coripata, Hacienda El Chorro, 1700 m, 3 Jul. 1930, *Buchtien 8050** (BAA, US); Nor Yungas, camino La Paz–Coroico, pasando Chuspipata, 2500 m, 16 Abr. 1983, *Beck 9158** (LPB, SI, US); near Sorata, Jun. 1860, *Mandon 1250* (BAA, US). COLOMBIA. Sin localidad, *Moritz 636* (US). **Huila:** La Plata, 2600 m, 12 Mar. 1939, *Sneidern 2112** (US). **Putumayo:** Sibundoy, 17

May 1935, *García Barriga 4569** (US). ECUADOR. **Azuay:** 71 km SW of Cuenca on road to Loja, 12 km N of Río León, 2000 m, 30 Mayo 1990, *Peterson & Judziewicz 9366* (US). **Bolívar:** just outside Fecundo Vela near a cornfield, 1880 m, 4 Mayo 1990, *Peterson & Annable 9021* (MO). **Chimborazo:** 6 km NE of Pallatanga, then 18.5 km W on road to Chillanes, then 17 km N on road to Cañil, 2700 m, 23 Mayo 1990, *Peterson et al. 9278* (MO). **Imbabura:** cercanías de Otavalo, Hda. Rosas, 2800–3000 m, 15 Mayo 1944, *Acosta Solís 8026* (US). **Loja:** Zamora–Huaico, Cordillera Oriental, 2600–2800 m, 2 Apr. 1951, *Acosta Solís 20517* (US); 18.4 km W of El Empalme on road to Celica, 1800 m, 3 Jun. 1990, *Peterson & Judziewicz 9494* (MO). **Archipiélago de Colón:** Albemarle Island, Tagus Cove Mt., 4000 ft., 26 Mayo 1932, *Howell 9574A* (US); Albemarle Island, Tagus Cove Mt., 2500 ft., 25 Mayo 1932, *Howell 9539* (US); Albemarle Island, Tagus Cove, 600–4000 ft., Mar. 1927, *Stewart 1320* (MO, US). **Pichincha:** Tambillo, 2700 m, 25 Mayo 1939, *Asplund 6446* (US); Tumbaco, 2400 m, 29 Mayo 1939, *Asplund 6577* (LIL, US); Quito, 2900 m, 5 Jul. 1952, *Acosta Solís 21169* (US). **Sin Provincia:** sin localidad, *Spruce 5943* (US), *Jameson 280* (US). PERU. **Apurímac:** Ollantay, Tambo, 3000 m, 6 Mayo 1915, *Cook & Gilbert 586* (US). **Cajamarca:** Contumazá, alrededores de Guzmango, 2500 m, 22 Mayo 1978, *Sagástegui & Mostacero 9088* (MO); Contumazá, Yetón, San Benito–Guzmango, 1800 m, 12 Jun. 1983, *Sagástegui & López 10544* (MO, US), *10550A* (MO). **Cuzco:** Urubamba, a 110 km de Cuzco en el camino, 13°00′S, 72°31′W, 2050 m, 21 Mayo 1988, *Núñez et al. 9146* (MO); ca. 5 km N of Aguas Calientes (km 116, railroad), ca. 2000 m, 7 Jun. 1977, *Solomon 3193* (MO); Convención, Amaybamba, 1800 m, Mayo 1949, *Marín 1480* (LIL); Calea, Piscal, 3000 m, Abr. 1944, *Marín 321* (LIL). **Huancavelica:** Tayacaja, alrededores de Salcabamba, 3200 m, 13 Abr. 1962, *Tovar 3614* (US). **Junín:** Acopalca, 4000 m, 20 Jul. 1945, *Infantes 402* (LIL); Huancayo, La Cabaña, near Huancayo, 3300 m, *Anderson 697* (US). **La Libertad:** Trujillo, Cerro Campana, 600 m, 14 Abr. 1953, *Angulo 2037* (US). **Lima:** Lomas de Atocongo, a 32 km de Lima, 300 m, 12 Oct. 1951, *Cerrate 832* (USM, US); Chancay, 100–700 m, Jul.–Oct. 1949, *Velarde Nuñez 2244* (US); Lurin, 23 Sep. 1923, *MacBride 5947* (US). **Lambayeque:** carretera Olmos–Marañón, entre Beatita de Humay y km 38, 150 m, 21 Mayo 1963, *López et al. 4048* (MO). **Piura:** Huancabamba, arriba de Palambla cerca de Canchaque, 1500–1600 m, 1 Mayo 1955, *Ferreyra 10834* (US). VENEZUELA. **Aragua:** prope Colonia Tovar, 1856/1857, *Fendler 1696* (MO, US). **Lara:** Morán, ± 1 km del caserío La Peña a 16.5 km de Humocaro Alto, 1800 m, 3 Oct. 1978, *Burandt Jr. V0385* (MO). **Trujillo:** Serranías de La Cristalina, 1900–2400 m, 28 Ago. 1941, *Tamayo 1859*** (US).

Paspalum penicillatum se distingue de *P. prostratum* por tener espiguillas elipsoides, de 1.2–1.9 mm de largo, 0.6–0.8 mm de ancho, con el dorso de la lemma inferior marcadamente surcado. Las espiguillas comúnmente se disponen en 2 series a lo largo de los racimos; excepcionalmente se observaron espiguillas uniseriadas en el espécimen *Solomon & Nee 14340* y en *Stewart 1320* y en 2 ejemplares de Venezuela (*Burandt Jr. V0385* y *Fendler 1696*).

Es esta una especie marcadamente variable en el tamaño de las plantas, encontrándose ejemplares con cañas de 0.10–0.20 cm (por ejemplo *Velarde Núñez 2244* posee una altura de 6–11 cm, con espiguillas de 1.2–1.4 mm de largo) hasta otros de 100–200 cm de largo (*Buchtien 8050*).

Los ejemplares pequeños (como *Velarde Núñez 2244*, *Beck 7219*, *Cerrate 832*, *Ferreyra 3898*, *4002*, *10834*, y *Peterson et al. 8754*), de 10–20 cm de alto, relacionan a *P. penicillatum* con *P. pygmaeum*, separándose *P. penicillatum* por poseer cañas geniculadas a decumbentes, arraigadas en los nudos inferiores, con láminas lanceoladas a ovado-lanceoladas, de 0.6–1 cm de ancho, con 6–17 racimos por inflorescencia.

En lo referente a la distribución altitudinal de *P. penicillatum* se hallaron ejemplares a bajas elevaciones únicamente en Ecuador, en el Archipiélago de Colón y en Perú, en los departamentos La Libertad, Lima, y Lambayeque, sobre la costa del océano Pacífico.

4. Paspalum prostratum Scribn. & Merr., U.S.D.A. Div. Agrostol. Bull. 24: 9. 1901. TIPO: México. Michoacán: low lands near Pátzcuaro, 9 Nov. 1890, *Pringle 3343** (holótipo, US-2855765; isótipo, P, fragmento, BAA). Figura 7.

Plantas anuales, con *cañas* geniculadas a decumbentes, arraigadas y ramificadas en los nudos inferiores, luego ascendentes, geniculadas, de 20–50 cm de alto; entrenudos de 2–13 cm de largo, cilíndricos a comprimidos, brillantes, glabros, estriados; nudos glabros, castaños. *Vainas* de 3–8 cm de largo, usualmente más cortas que los entrenudos, esparcidamente pilosas hacia los márgenes y el ápice. *Lígulas* de 0.9–2 mm de largo, castañas, cortamente pilosas en la cara abaxial; cuello glabro. *Láminas* lanceoladas, de 3–10 cm de largo por 0.3–1 cm de ancho, herbáceas, de base redondeada, no pseudopecioladas, y ápice acuminado, esparcidas a densamente papiloso-pilosas o glabrescentes; nervio medio prominente, los márgenes ciliados o no, escabriúsculos. *Pedúnculos* de 3–15 cm de largo, angulosos, delgados, glabros. *Inflorescencias* angostamente elipsoides, de 5–20 cm de largo, 1–2 cm de ancho; eje principal anguloso, liso a escabriúsculo, terminando en ocasiones en una prolongación estéril; pulvínulos escabriúsculos a cortamente pilosos; ramificaciones 4–10(–20), alternas, las basales distantes, terminando

en una prolongación estéril; raquis de las ramificaciones de 1–5 cm de largo, 1.8–2.5 mm de ancho, glabro a cortamente pubescente en la cara adaxial, con la vena media marcada, blanca, flexuosa, la cara abaxial cortamente pubescente a glabra, los márgenes escabriúsculos; pedicelos de 0.6 mm de largo, aplanados, escabriúsculos. *Espiguillas* ovoides a obovoides, de (1.9–)2.1–2.7 mm de largo, 1–1.2 mm de ancho, distanciadas, uniseriadas, ocasionalmente en dos series, glabras, de ápice agudo, cerradas a la madurez, verde-pálidas o con tintes purpúreos. *Gluma superior* y *lemma inferior* subiguales, tan largas como la espiguilla, lisas, glabras, delgadas, 3-nervias; lemma inferior suavemente surcada en la región central. *Antecio superior* elipsoide, de (1.8–)2.1–2.4 mm de largo, 0.9–1 mm de ancho, de ápice agudo, pálido, crustáceo, persistente, brillante, la lemma y pálea papilosas, con papilas simples notorias y micropelos en toda la superficie; lemma 3-nervia; pálea 2-nervia; lodículas 2, de 0.3 mm de largo, hialinas; estambres 3, anteras de 0.6–1 mm de largo, amarillo-ocráceas. *Cariopsis* ovoide a elipsoide, de 1.4–1.7 mm de largo, 0.8–0.9 mm de ancho; hilo oblongo; embrión ⅓ del largo de la cariopsis.

Nombre vulgar. "Grama," "maicillo," "tomate," "nudillo blanco" (Colombia); "conejito" (Venezuela).

Distribución y ecología. Habita en México, Venezuela, Colombia, Ecuador, y Perú, en bosques húmedos y bordes de camino sobre suelos arcillosos o arenosos, entre 1500 y 2850(–4000) m.

Material adicional examinado. BOLIVIA. **Cochabamba:** Ayopaya, cuenca del Río Tambillo, Estancia Chuñawi, 2840 m, 30 Mar. 1989, *Baar 114* (LBP). COLOMBIA. **Antioquia:** Caldas, 24 Nov. 1930, *Archer 1717* (US); Medellín, 23 Sep. 1927, *Toro 675* (US); El Corcovado, Ene. 1946, *Hno. Daniel 3845* (COL, US); a 1 km al norte de Santa Rosa de Osos, 2600 m, 25 Sep. 1948, *Araque Molina & Barkley 18A025* (COL, US), *18A040* (COL, LIL, US); alrededores del Río Negro, 2120 m, 1 Nov. 1947, *Correa et al. 1563* (US). **Boyacá:** Tota, 1 Sep. 1951, *Yepes-Agredo 3132* (COL); Ventaquemada, 2 Jun. 1971, *Guarín & Villarreal 951* (COL); Pajarito, Corregimiento de Corinto, 2200 m, 10 Oct. 1967, *Lozano et al. 835* (COL). **Cauca:** Coconuco, 2300–2500 m, 17/18 Jun. 1922, *Killip 6861* (US); Parque Nacional de Puracé, 3000 m, 28 Dic. 1988, *Ortíz 1281* (COL). **Cundinamarca:** Choachí, 2 Jun. 1959, *García-Barriga 17164* (COL, US); Bogotá, *Idinael 278* (US). **Nariño:** Las Lajas, 5 Ago. 1939, *García-Barriga 7836*** (COL). **Santander:** Carretera Bucaramanga–Cúcuta, 1950 m, 29 Sep. 1966, *Robinson Jr. & Beltrán 3131, 3133* (US); vicinity of California, 3000 m, 11/27 Jun. 1927, *Killip & Smith 16936* (US). ECUADOR. **Loja:** 4 km E of Guachanamá on steep road down to Macará Catacocha Highway, 2530 m, 3 Jun. 1990, *Peterson & Judziewicz 9515* (MO). **Zamora-Chinchipe:** 21–22 km S of Yangana on road to Zumba, at pass of Cordillera Occidental, 2750–2850 m, 31 Mayo 1990, *Peterson & Judziewicz 9420* (MO). MEXICO. **Hidalgo:** Trinidad Iron Works, 5800 ft., 15 Sep. 1904, *Pringle 8891** (LIL, MO, SI). **Michoacán:** al W de Morelia, 7 Oct. 1909, *Arsène s.n.* (LIL 38697, 38708), *Arsène 3129* (MO, US). PERU. **Apurímac:** Abancay, 12 Jun. 1946, *Infantes 891* (LIL). **Cajamarca:** Contumazá, alrededores de Yetón, 2200 m, 1 Mayo 1981, *Sagástegui et al. 9766* (MO); Contumazá, alrededores de Guzmango, 2600 m, 1 Mayo 1981, *Sagástegui et al. 9768* (MO). **Cuzco:** Calca, 3000 m, Abr. 1944, *Moreno 321* (BAA); Calca, alturas del Tucson, 3400 m, Feb. 1945, *Moreno 511* (BAA). **Junín:** Acolla, 3600 m, 9 Abr. 1960, *Kunkel 605* (BAA); Huancayo, 3500 m, 15 Mayo 1960, *Kunkel 604* (BAA). **Sin Departamento:** sin localidad, *Brunnell 787* (MO). VENEZUELA. **Lara:** Jiménez, 4–5 km S of Sanare, 69°38′W, 9°42′N, 1550 m, 28 Oct. 1982, *Davidse & González 21366* (MO, SI); Morán, lado del camino entre La Peña y las "filas de las vacas" a 16.5 km de Humocaro Alto, 1750 m, 3 Oct. 1978, *Burandt Jr. V0378* (MO). **Mérida:** Mérida, Sep. 1952, *Humbert 26591* (COL); Andrés Bello, steep slopes between Quebrada El Trigal and Quebrada Zerpa, between Mirabel and San Benito, 10–14 km SE of La Azulita, 8°40–41′N, 71°25/26′W, 1600 m, 1 Jul. 1980, *Davidse & González 18892* (MO); 2 km SW of Santo Domingo along Highway to Mérida, 2100 m, 19 Nov. 1971, *Davidse 3210* (MO); Cañada del Cedro, arriba de Bailadores, 2300 m, 20 Sep. 1942, *Tamayo 2313* (US). **Táchira:** Quebrada Agua Azul, S of El Reposo, 14 km SE of Delicias, 7°31′N, 72°24′W, 2150–2300 m, 22/23 Jul. 1979, *Steyermark & Liesner 118655* (MO); Junín, Southern slopes of Cerro San Isidro, directly N of El Reposo, above Hacienda Bella Vista, Quebrada Agua Caliente and tributaries, 72°25′W, 7°34′N, 2000 m, 13/14 Nov. 1982, *Davidse & González 22249* (MO); Junín, Quebrada Agua Blanca, near La Rochela, along Delicias–Rubio Highway, 72°26′W, 7°35′N, 1850–2000 m, *Davidse & González 22323* (MO). **Trujillo:** Carache, vía Páramo de Turmal, 9°36′N, 70°9′W, 2200 m, 1 Nov. 1987, *Rivero & Díaz 1385* (MO).

Paspalum prostratum posee las espiguillas comúnmente distribuidas en una serie a lo largo de los racimos. Ocasionalmente se ha observado la presencia de espiguillas distribuidas en 1–2-series en los ejemplares *Davidse & González 21366, Robinson Jr. & Beltrán 3131, 3133, Arsène 3129, Hinton 2393,* y *Pringle 8891*. Los ejemplares *Baar 114* y *Cárdenas 3414* se diferencian por tener espiguillas en 2 series. A su vez el ejemplar tipo de *P. prostratum* posee algunos racimos con espiguillas en 2-series y espiguillas de 1.9 mm de largo.

El especímen *de Alba 53-66* incluye plantas de 8 cm de alto, asemejándose en este carácter a *Paspalum pygmaeum*. Se distingue de esta última especie por tener espiguillas 1-seriadas de 2.4–2.5 mm de largo. Posiblemente su porte esté condicio-

FIGURA 9. Distribución de *P. pygmaeum*, *P. rugulosum*, *P. sodiroanum*, y *P. soukupii*.

nado por la altura donde crece en Venezuela: Pico del Aguila a 4000 m de altura.

5. Paspalum pygmaeum Hack., Repert. Sp. Nov. Regni Veg. 11: 18. 1912. *Paspalum pygmaeum* Hack. var. *genuinum* Hack., Repert. Sp. Nov. Regni Veg. 11: 18. 1912, nom. inval. TIPO: Bolivia. La Paz: in altiplanitie prope La Paz, 4100 m, 1907, *Buchtien 859*** (holótipo, W no visto; isótipo, US-1099133). Figuras 9, 10.

Paspalum haenkeanum Nees var. *minimum* Nees, Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 19(Suppl. 1): 6. 1841. *Paspalum minimum* Meyen, Reise Erde 1: 484. 1834, nom. nud. TIPO: Perú. Maravillas, 1600 ft., Abr., *Meyen s.n.* (holótipo, B no visto, fragmento, US-2942511).

Paspalum prostratum Scribn. & Merr. var. *pygmaeum* Scribn. & Merr., U.S.D.A. Div. Agrostol. Bull. 24: 9. 1900. TIPO: México. Distrito Federal: Pedregal, valley of Mexico, 8500 ft., 30 Sep. 1896, *Pringle 7167* (holótipo, US-741449).

Paspalum pygmaeum Hack. var. *glabrescens* Hack., Repert. Sp. Nov. Regni Veg. 11: 18. 1912. TIPO: Bolivia. La Paz: in altiplanitie prope La Paz, 4100 m, 7 Abr. 1932, *Buchtien 2490* (holótipo, W no visto, fragmento, US-2942510; isótipos, MO-1041615, US-1099134, fragmento, US).

Paspalum lorentzianum Mez, Repert. Sp. Nov. Regni Veg. 15: 29. 1917. TIPO: Argentina. Salta: Los Potreros al pié del Nevado del Castillo, 18 Mar. 1876, *Lorentz & Hieronymus 180* (holótipo, B no visto, fragmentos, BAA, US-2855822).

Plantas anuales, cespitosas, arrosetadas, con *cañas* paucinodes de 1–8(–15) cm de alto, densamente ramificadas en los nudos inferiores; entrenudos de 0.5–1.5 cm de largo, teretes, híspidos; nudos castaños, glabros. *Vainas* de 0.8–3 cm de largo, densa a esparcidamente pilosas, los márgenes ciliados. *Lígulas* de 0.6–1 mm de largo, hialinas, con la cara abaxial cortamente pilosa. *Láminas* linear-lanceoladas, de 1–5.5 cm de largo, 0.15–0.4 cm de ancho, de base redondeada, no pseudopecioladas, y ápice agudo, híspidas a esparci-

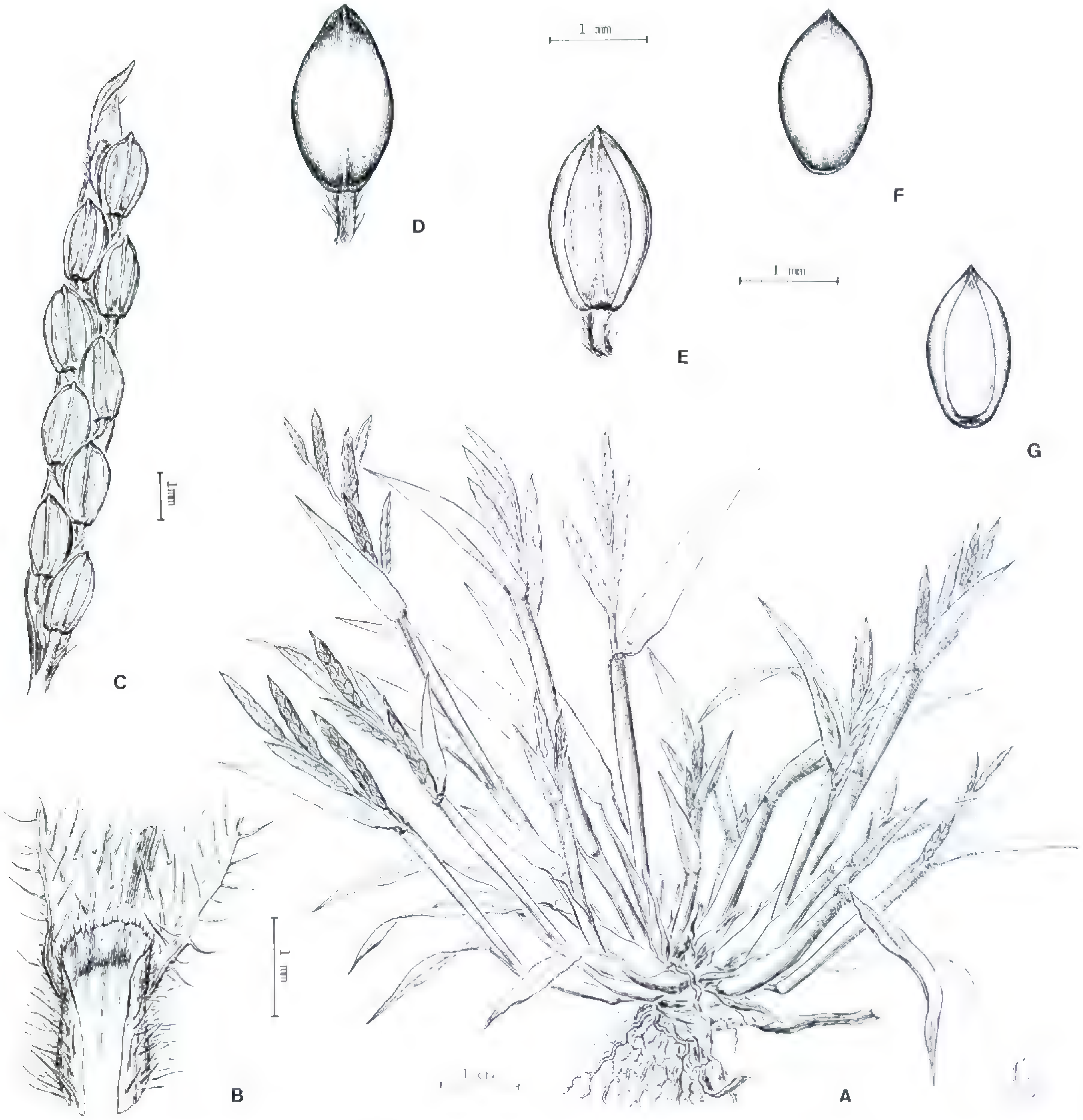


FIGURA 10. *Paspalum pygmaeum* Hack. (A de *Beck & Seidel 14512*, B–G de *Beck & Seidel 14567*).—A. Hábito.—B. Detalle de la región ligular.—C. Racimo de la inflorescencia.—D. Espiguilla vista del lado de la gluma superior.—E. Espiguilla vista del lado de la lemma inferior.—F. Antecio superior visto del lado de la lemma superior. G. Antecio superior visto del lado de la pálea superior.

damente pilosas, los márgenes ciliados. *Pedúnculos* hasta de 5 cm de largo, cilíndricos, glabros. *Inflorescencias* subexertas, de 1.5–3 cm de largo, 1–2 cm de ancho; eje principal aplanado, surcado, con pelos hacia los bordes o glabro; pulvínulos cortamente pilosos; ramificaciones 2–6, alternas; raquis de las ramificaciones de 0.4–1.5(–2) cm de largo, 0.4–2 mm de ancho, de ápice agudo y terminando en una prolongación estéril, la cara adaxial glabra a pubescente, el nervio medio en zig-zag, la cara abaxial cortamente pilosa, con el nervio medio aplanado y piloso, los márgenes glabros o ciliados; pedicelos de 0.3–0.8 mm de largo, aplanados. *Espiguillas* ovoides a elipsoides, de 1.5–2.1 mm de largo, 0.7–1.2 mm de ancho, biseriadas, glabras, de ápice agudo, cerradas a la madurez, verde-pálidas. *Gluma superior* y *lemma inferior* subiguales, tan largas como la espiguilla, lisas, glabras, 3-nervias; gluma superior cubriendo con los bordes a la lemma inferior; lemma inferior de dorso plano. *Antecio superior* elipsoide, de 1.4–1.9 mm de largo, 0.6–0.9 mm de ancho, crustáceo, pajizo, no caedizo; lemma y pálea con papilas simples en toda la superficie, con cuerpos de sílice exfoliados

hacia el ápice de la lemma; estambres 3, anteras de 1.1 mm de largo; lodículas 2, de 0.2 mm de largo. *Cariopsis* de 1.2 mm de largo, 0.7 mm de ancho, pajizo; hilo punctiforme; embrión ⅓ del largo de la cariopsis.

Nombre vulgar. "Pacha hueta," "sara-sara" (en Perú).

Distribución y ecología. Especie de distribución disyunta, habitando en México, en el Distrito Federal y el estado de Morelos y en Sudamérica en Colombia, Venezuela, Perú, Bolivia, y la República Argentina, en laderas pedregosas graminosas, entre los 2600 y 4600 m.

Material adicional examinado. ARGENTINA. **Catamarca:** Ambato, Cerro El Manchado, 3100 m, 24 Feb. 1967, *Türpe 1156* (LIL). **Jujuy:** Tumbaya, Cerro Chañi, 3500 m, 30 Ene. 1929, *Venturi 8392*,*** (US); sin localidad, 1940, *sin colector, ex SI 16452* (BAA); Santa Ana, 29 Feb. 1940, *Burkart & Troncoso 11670* (BAA). **Tucumán:** Chicligasta, Estancia Las Pavas, Puesto La Cascada, 2700 m, 11 Mar. 1924, *Venturi 3025*,*** (LIL, US), 16 Mar. 1924, *Venturi 3041* (US); La Ciénaga, 2900 m, 7 Feb. 1933, *Parodi 11075** (US); Tafí, La Ciénaga, 2500 m, 2 Abr. 1926, *Schreiter 4473* (LIL); Peñas Azules, 3400 m, 28 Ene. 1933, *Parodi 10884* (BAA). BOLIVIA. **Cochabamba:** Ayopaya, 10 km al NW de Independencia, 3000 m, 10 Mayo 1988, *Beck & Seidel 14512* (LPB); Ayopaya, camino de Independencia hacia Kami, 8 km antes de llegar a Kami, 3750 m, 11 Mayo 1988, *Beck & Seidel 14567** (LPB); Quillacollo, camino Sipe-Sipe–Lipichi, 3800 m, 9 Abr. 1990, *Hensen 736** (LPB). **La Paz:** Murillo, Valle del Río Zongo, 14.8 km N de la cumbre, 16°12'S, 68°07'W, 3900–4000 m, 11 Abr. 1987, *Solomon et al. 16513* (MO); Nor Yungas, Pongo, 27 Dic. 1923, *Hitchcock 22772* (US); Murillo, La Paz to Unduavi, 12 km E of La Cumbre, 3800 m, 3 Abr. 1981, *Renvoize & Cope 4162** (LPB, MO); Omasuyos, Tiquina 6 km hacia La Paz, 3900 m, 24 Feb. 1980, *Beck 2923** (LPB, SI); Ingavi, granja de Cordepaz en Huacullani, 3950 m, 30 Ene. 1980, *Beck 2452** (LPB); Murillo, 15.6 km above (SW) Cuticucho hydroelectric plant, 16°13'S, 68°07'W, 4100 m, 17 Abr. 1985, *Solomon 13377* (LPB, MO); Ingavi, cantón Jesús de Machaca, comunidad Titicani-Tacaca, a 20 km de Guaqui, 4160 m, 8 Abr. 1989, *Villavicencio 525, 810* (LPB), *819* (LPB, SI), 12 Mar. 1989, *Villavicencio 288* (SI); Ingavi, 8 km NE of Taraco, 3820–3900 m, 31 Mar. 1981, *Renvoize & Cope 4130* (LPB), *4142* (LPB, MO); Murillo, 17 km al E de La Cumbre por el camino a Unduavi, 4.2 km al W de Unduavi, 16°19'S, 67°55'W, 3350 m, 11 Abr. 1988, *Solomon 18263* (MO, SI, US); Amasuyos, a la altura de Laja, sobre la ruta La Paz–Huarina, ± 3900 m, 28 Feb. 1950, *Krapovickas & Fuchs 7061* (BAA, LIL). **Sin Departamento:** Talca Chugiaguillo, Abr. 1890, *Bang 808* (MO, US 3 hojas). COLOMBIA. **Cauca:** zona de las Tres Cruces, en los alrededores de Popayán, 6 Nov. 1948, *Agredo et al. 18Ca066* (US). MEXICO. **Morelos:** Lago de Zempoala, 3000 m, 7 Oct. 1951, *Matuda 25584* (US). PERU. **Ancash:** Bolognesi, Tanás, cerro al S de Chiquián, 3900 m, 26 Abr. 1952, *Cerrate 1375** (LPB, US); Huaraz, Huascarán National Park, Quebrada Rajucolta, 77°22'W, 9°32'S, 4000–4150 m, 17/18 Abr. 1986, *Smith et al. 12160* (MO). **Apurímac:** Hacienda Seccsecka, 3200 m, 3 Mar. 1946, *Vargas 5697* (US). **Ayacucho:** Pampalca, between Huanta and Río Apurímac, 3200 m, 4/5 Mayo 1929, *Killip & Smith 22215, 22216a* (US). **Cuzco:** Aguas Calientes, 4150 m, 11 Abr. 1943, *Vargas 3310* (BAA); Paruro, alturas de Jaurisque, 3650 m, Abr. 1957, *Vargas 11574* (US); Valle de Anta, 3280 m, 1 Sep. 1920, *Stordy s.n.* (US 1127272); Calca, Pisac, 3300 m, Feb. 1949, *Marin 1341* (LIL); Calca, Abra de Amparaes, 4250 m, 10 Mayo 1075, *Chávez 3324* (MO); Yauri, entre el Río Salado y la carretera a Tintaya, 3900 m, 29 Mar. 1987, *Núñez & Urrunaga 7678* (MO). **Huancavelica:** Huancavelica, Patacancha, pajonal entre Conaica y Luquia, 4000 m, 8 Abr. 1961, *Tovar 3135* (US, USM); Huancavelica, Santa Rosa, abajo de Huancavelica, 3600–3650 m, 11 Mayo 1958, *Tovar 2983* (US); Tayacaja, Hda. Huari, 3700–4200 m, Mar. 1955, *Salaverry 122* (US). **Junín:** La Quinua, 12,000 ft., 14 Mayo 1922, *MacBride & Featherstone 2030* (US). **Lima:** Huancayo, cerros al E de Huancayo, 3900 m, 2 Mayo 1954, *Tovar 2164* (USM); Huancayo, Acopalca, 20 Jul. 1945, *Infantes 424* (LIL). **Puno:** entre Llave y Mazo Cruz, 3900 m, 9 Mar. 1966, *Tovar 5301* (USM); Cuquibambilla, 3900–4000 m s.m., 19/21 Abr. 1925, *Pennell 13377* (US); Carabaya, Fauchinta Allic-capac, 4600 m, 1 Abr. 1948, *Vargas 7156* (US); Huerta, N of Puno, 4000 m, 2 Mar. 1957, *Ellenberg 474* (US); Pucará, *Weberbauer 415* (US). **Sin Departamento:** La Raya, 4300 m, 4 Abr. 1954, *Ranh-Hirsch P677* (US); sin localidad, *Weddell 4423, 4490* (US); Quebrada de Iscos, a 6 km de Concepción, 3300 m, 9 Mar. 1947, *Ochoa 16* (US); Salcanbay, 3200 m, *Ranh-Hirsch P1417* (US); Cerro San Cristóbal, 3500 m, 8 Jul. 1977, *Evangelista s.n.* (MO 2636402); sin localidad, *Herrara 1922* (US). VENEZUELA. **Mérida:** Sierra de Santo Domingo, Páramo de Mucubají, alrededores de la Laguna Grande, 3560–3600 m, 19 Nov. 1959, *Barclay & Juajibioy 9551* (MO); Páramo Santo Domingo, 3200 m, 3 Oct. 1956, *Vareschi & Vareschi 5775* (MO); Rangel, along road Sto. Domingo–Apartaderos, ca. 3000 m, 29 Oct. 1985, *van der Werff 7701* (MO); Sierra Nevada, Páramo de Mucubají, Finca La Corcovada, naciente del Río Santo Domingo, 3200 m, 26 Sep. 1966, *Schulz & Bubberman 658* (MO, US) *658a* (US); Parque Nacional Sierra Nevada, Páramo Mucubají, SE de la entrada, 3600 m, 11 Oct. 1980, *Briceño & Adamo 225* (MO, US); Páramo Mucubají, cerca de la Laguna Grande, 3500 m, 7/8 Oct. 1965, *Schulz et al. 99* (US).

Especie afín a *P. prostratum*, de la que se distingue por ser plantas usualmente arrosetadas, de 1–8(–15) cm de alto, con espiguillas biseriadas, de 1.5–2.1 mm de largo y láminas linear-lanceoladas de 1.5–5 cm de largo por 0.15–0.4 cm de ancho.

Chase (1929) incluyó a *Paspalum prostratum* var. *pygmaeum* en la sinonimia de *P. prostratum*. El análisis del material tipico depositado en US mostró que el mismo consiste de 3 plantas de 3–5 cm de alto, con espiguillas biseriadas de 1.7(–2) mm de largo, razón por la cual se incluye a *P. prostratum* var. *pygmaeum* en la sinonimia de *P. pygmaeum*.

FIGURA 11. Distribución de *P. racemosum* y *P. reclinatum*.

Paspalum pygmaeum se caracteriza por sus plantas de pequeño porte, con hábito arrosetado. Ocasionalmente se analizaron ejemplares con cañas alargadas, similares a *P. penicillatum*, pudiéndose citar entre los mismos a *Cerrate 1375*, *Castillón s.n.*, y *Meyer 14913*; los mismos se distinguen de *P. penicillatum* por tener láminas linear-lanceoladas, inflorescencias con un menor número de racimos, 2–3, y espiguillas no surcadas, mayores, de 1.6–2.1 mm de largo.

6. Paspalum racemosum Lam., Tabl. Encycl. 1: 176. 1791. TIPO: Perú. "Comm. D. Boutelou" (holótipo, P, fragmento, BAA, fragmento y foto, US-2855976; isótipo, P). Figuras 11, 12.

Paspalum stoloniferum Bosc, Trans. Linn. Soc. London 2: 83, tab. 16. 1794. *Paspalanthium stoloniferum* (Bosc) Desv., Opusc. Sci. Phys. Nat.: 59. 1831. *Maizilla stolonifera* (Bosc) Schlecht. Bot. Zeitung (Berlin) 8: 605. 1850. TIPO: Perú. "H. in Perua" (holótipo, PAD no visto; fragmento, US-80053; isótipos, M no visto, P, fragmento, BAA, fragmento y foto, US-80053).

Milium latifolium Cav., Icon. 3: 37, pl. 273. Dic. 1795 Ene. 1796, hom. illeg., non Moench, 1794. TIPO: Perú. "Habitat in Peru" (holótipo, MA? no visto; isótipo, PAD no visto, fragmento y foto, US-80053; isótipos, B no visto, fragmento, US-80053; P no visto, fragmento, US-80053).

Paspalum purpureum Ruíz & Pav., Fl. Peruv. 1: 47. 1798. TIPO. Perú. "Habitat in Peruviae cultis, et in Hort. Reg. Matritensis singulis annis coliture seminibus missis per nosmetipsos" (tipo no visto).

Paspalum biglume Steud., Syn. Pl. Glumac. 1: 24. 1853. TIPO: "Sub Paspalum stoloniferum Bosc cultum in H. Goetting" (holótipo, P no visto, fragmento, US-2942155).

Paspalum manabiense Mez, Repert. Sp. Nov. Regni Veg. 15: 30. 1917. TIPO: Ecuador. Manabí: Hacienda El Recreo, *Eggers 14965* (holótipo, B, fragmento, US-80090; isótipos, M no visto, P, fragmentos, MO-1042314, US-80090, US-824049).

Plantas anuales, con *cañas* de 30–100 cm de alto, geniculadas, ascendentes, ramificadas y arrai-

Quebrada Los Conejos and adjacent ridge, ca. 25 km SE of Cherrelique, 04°09'S, 80°37'W, 600–850 m, 10 Jun. 1987, *Gentry & Díaz 58313* (MO). **Sin Departamento:** Santa Clara, 18 Jul. 1914, *Rosee & Rose 18742* (US); Canruru, Mayo 1879, *Savatier s.n.* (US); Cerro de la Brea, Jul. 1926, *Haught 108* (US); sin localidad, *Anderson s.n.* (US).

Paspalum racemosum se distingue de *P. flavum* por tener racimos rectos, espiguillas de 2.1–3 mm de largo, de ápice piloso, con gluma superior y lemma inferior transverso-rugosa, con papilas infladas hacia la porción basal de ambas brácteas.

7. **Paspalum reclinatum** Chase, J. Wash. Acad. Sci. 33(10): 317. 1943. TIPO: Colombia. Cauca: Cordillera Occidental, Cerro de Munchique, vertiente W, en la Hoya del Río Tambito, 2000–2500 m, 16 Jul. 1939, *Pérez Arbeláez & Cuatrecasas 6211*** (holótipo, US-1795921; isótipo, US-1795920). Figura 11.

Plantas anuales, con *cañas* decumbentes a geniculadas, arraigadas y ramificadas en los nudos inferiores, cañas floríferas ascendentes de 50–100 cm de alto; entrenudos de (1.6–)7–11 cm de largo, huecos, glabros, estriados; nudos glabros o ligeramente pubescentes. *Vainas* de 4.5–11 cm de largo, más cortas o más largas que los entrenudos, con pelos papilosos, delgados, hacia el ápice o sin los mismos, los márgenes membranáceos, ciliados hacia la porción superior, glabros en el resto de la superficie. *Lígulas* de 0.4–1.2 mm de largo, castañas, pubescentes en la cara abaxial. *Láminas* lanceoladas, de 4–15 cm de largo, 0.4–1.2 cm de ancho, pseudopecioladas, pseudopeciolo de 1.5–2 mm de largo, cortamente pubescente en ambas caras; láminas de base angostada, glabras, los márgenes escabrosos, el nervio medio manifiesto. *Pedúnculos* hasta de 20 cm de largo, angulosos, glabros, verdosos. *Inflorescencias* terminales exertas, de 8–17 cm de largo, 1.5–4 cm de ancho; eje principal anguloso, verdoso, escabroso, terminando en un racimo; pulvínulos densamente pilosos; ramificaciones 7–12, aproximadas o distantes; raquis de las ramificaciones de 1.2–4.5 cm de largo, 1–2 mm de ancho, terminando en una espiguilla u ocasionamente en una prolongación estéril, verdoso, cortamente pubescente en la cara abaxial, glabro en la cara adaxial, el nervio medio blanquecino, ligeramente flexuoso en la cara adaxial, interiormente alado, escabroso, márgenes del raquis escabérulos; pedicelos de 0.5–1.3 mm de largo, aplanados. *Espiguillas* ovoides, de 2.3–3 mm de largo, 1 mm de ancho, biseriadas, cerradas a la madurez. *Gluma superior* y *lemma inferior* lisas a ligeramente corrugadas en la porción inferior, subiguales, agudas, superando 0.5 mm en largo al antecio superior, glabras, 3-nervias, con los nervios manifiestos. *Antecio superior* elipsoide, de 2.1–2.5 mm de largo, 0.7–1 mm de ancho, pajizo a la madurez, crustáceo, brillante; lemma 1–3-nervia, con cuerpos de sílice exfoliados hacia el ápice; pálea con papilas simples, tenues y cuerpos de sílice exfoliados hacia el ápice; lodículas 2, de 0.3 mm de largo, truncadas; estambres 3, anteras de 1.5 mm de largo, anaranjadas o purpúreas. *Cariopsis* obovoide, de 1.3–1.7 mm de largo, 0.7 mm de ancho, castaño; hilo oblongo, ⅓ del largo de la cariopsis; embrión poco menos de ½ del largo de la cariopsis.

Distribución y ecología. Costa Rica, Colombia y Ecuador, en ambientes semiumbrosos en bosques secundarios, en lugares húmedos entre 1900 y 2500 m.

Material adicional examinado. COLOMBIA. **Boyacá:** Río Pómeca, below Arcabuco, 7 Mar. 1984, 2400 m, 7 Mar. 1984, *Wood 4305** (COL). **Cauca:** de El Tambo a Munchique, 2370 m, 28 Jun. 1989, *Zuloaga 4259* (COL, SI). COSTA RICA. **Cartago:** Canyon of Río Grande de Orosi, S of Tapanti, 1500–1600 m, 9 Jun. 1973, *Pohl 12882* (MO). **San José:** Quebrado Corralillo, 2 km E of Rancho Redondo, 2100 m, 7 Feb. 1969, *Pohl & Davidse 11702** (US). ECUADOR. **Bolívar:** San Jacinto de la Unión, ca. 2500 m, 14 Ago. 1939, *Asplund 8242* (MO); below San Jacinto de la Unión, ca. 2300 m, 15 Ago. 1939, *Asplund 8317* (MO). **Pichincha:** Saloya, 7 km towards Quito, ca. 2500 m, 11 Sep. 1939, *Asplund 8635* (MO); carretera Sto. Domingo–Quito, 13 km al oeste del paso, 0°26'S, 78°38'W, 3000 m, 21 Jun. 1982, *Balslev 2763* (MO, US).

Paspalum reclinatum se caracteriza por poseer un pseudopecíolo breve y pubescente, espiguillas largamente elipsoides solitarias y gluma superior y lemma inferior 3-nervias, sobrepasando usualmente en largo al antecio superior. En la mayoría de los ejemplares se ha observado, tal como indica Chase en la descripción original, que los racimos terminan en una espiguilla; excepcionalmente se han hallado los racimos finalizando en una prolongación estéril en *Zuloaga 4259*.

8. **Paspalum rugulosum** Morrone & Zuloaga, sp. nov. TIPO: Ecuador. Chimborazo: Paso Redondo, 17.5 km SW of San Juan de Velasco on road to Pallatanga, 2430 m, 22 Mayo 1990, *Peterson, Judziewicz, King & Jorgensen 9249** (holótipo, US-3237226; isótipo, MO-3853357). Figuras 9, 13.

Paspalo reclinato Chase simile sed rhachidibus angustioris, glumis superis marginibus corrugatis et anthoeceis superis cartilagineis differt.

Annuum, glabrum; ligula 1.2–1.7 mm longa; laminae lanceolatae ad ovato-lanceolatae 2–6.5 cm longae, 0.4–1 cm latae; pseudopetiola usque 1 mm longa; racemi 6–

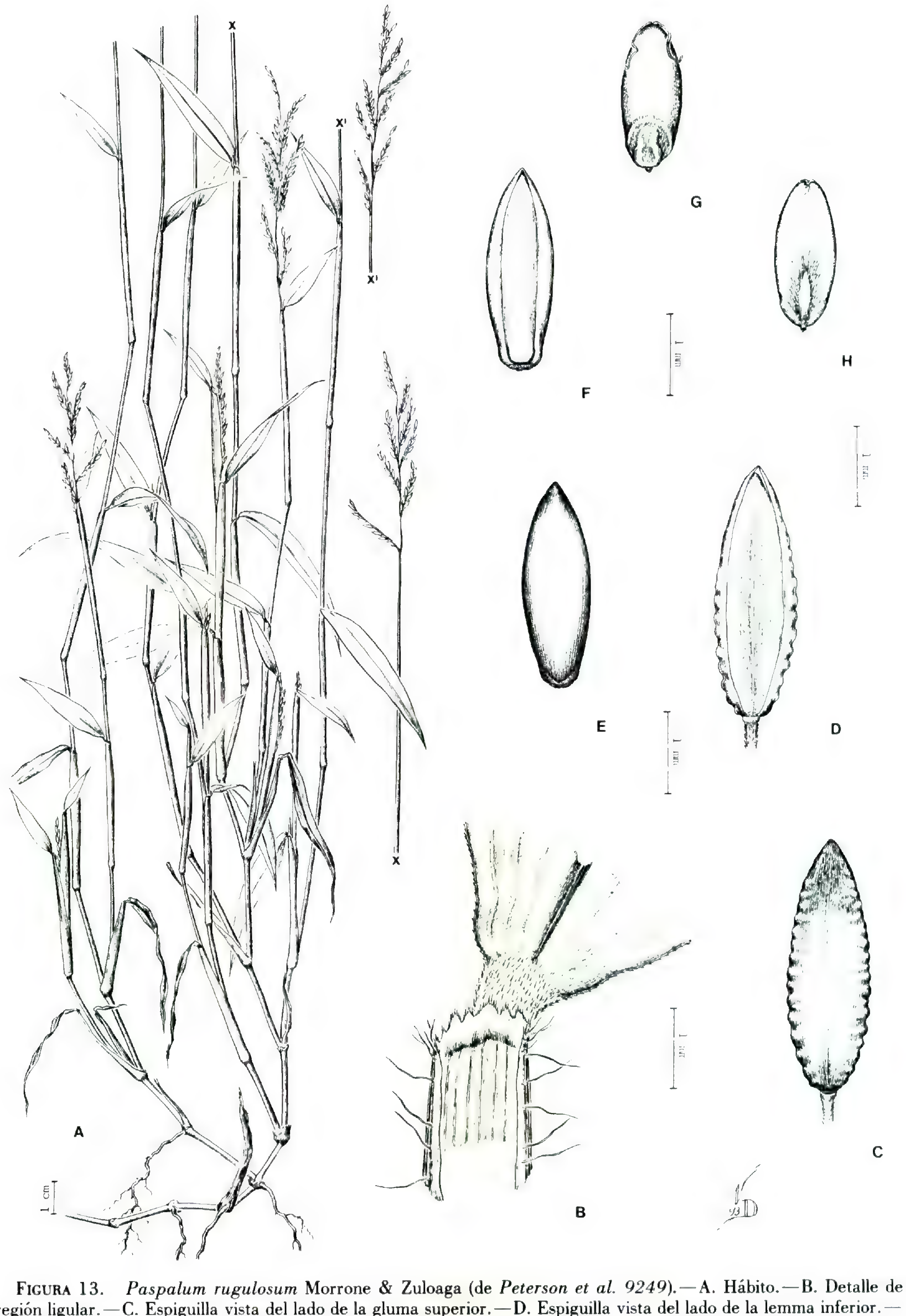


FIGURA 13. *Paspalum rugulosum* Morrone & Zuloaga (de *Peterson et al. 9249*).—A. Hábito.—B. Detalle de región ligular.—C. Espiguilla vista del lado de la gluma superior.—D. Espiguilla vista del lado de la lemma inferior.—E. Antecio superior visto del lado de la lemma superior.—F. Antecio superior visto del lado de la pálea superior.—G. Cariopsis, vista escutelar.—H. Cariopsis, vista hilar.

10, 1–3.5 cm longi; rhachis 0.5–0.8 cm lata; spiculae solitariae, longe ellipsoideae, marginibus corrugatis, 2.7–3 mm longae, 0.9–1.1 mm latae, glabrae; glumae superae et lemmae inferiores subaequales, 3-nerviae.

Plantas anuales, con *cañas* prostradas, arraigadas y ramificadas en los nudos inferiores, luego geniculadas a erectas, de 25–55 cm de alto, delgadas; entrenudos de 3–13 cm de largo, cilíndricos a comprimidos, glabros, violáceos; nudos glabros, castaños. *Vainas* más cortas que los entrenudos, de 2.5–7 cm de largo, verdosas, glabras, con los bordes membranáceos, largamente pestañosos. *Lígulas* de 1.2–1.7 mm de largo, con la cara adaxial glabra, cara abaxial cortamente hirsuta; cuello glabro. *Láminas* ovado-lanceoladas a lanceoladas, de 2–6.5 cm de largo, 0.4–1 cm de ancho, glabras, pseudopecioladas, pseudopecíolo hasta de 1 mm de largo, cortamente híspido, base de la lámina redondeada y ápice agudo, con los bordes escabriúsculos, el nervio medio manifiesto. *Pedúnculos* exertos, de 10–19 cm de largo, cilíndrico-aplanados, glabros, lisos, verdosos o con tintes violáceos. *Inflorescencias* exertas, de 4–7 cm de largo, 1–3 cm de ancho; eje principal anguloso, glabro, escabriúsculo sobre las aristas; pulvínulos cilíndricos, castaños, cortamente híspidos; ramificaciones 6–10, ascendentes, escasamente divergentes, alternas a subopuestas; raquis de las ramificaciones de 1–3.5 cm de largo, 0.5–0.8 mm de ancho, plano, cortamente alado, verdoso, con la cara adaxial glabra, la abaxial escabrosa, terminando en una espiguilla desarrollada, los márgenes escabrosos; pedicelos breves de 0.3–0.7 mm de largo, escabriúsculos. *Espiguillas* largamente elipsoides, de 2.7–3 mm de largo, 0.9–1.1 mm de ancho, de ápice agudo, biseriadas, no imbricadas, cerradas a la madurez, verdes pálidas o con tintes violáceos, con los márgenes fuertemente corrugados. *Gluma superior* y *lemma inferior* subiguales, tan largas como la espiguilla, delicadas, membranáceas, 0.4–0.6 mm más largas que el antecio superior, 3-nervias, con los nervios verdosos. *Antecio superior* largamente elipsoide, de 2.3–2.4 mm de largo, 0.7 mm de ancho, de ápice agudo, cartilaginoso, brillante, glabro, pajizo, con tintes violáceos a violáceos a la madurez; lemma lisa, no papilosa, con cuerpos de sílice exfoliados hacia el ápice; pálea finamente papilosa, con papilas simples en toda su superficie y cuerpos de sílice exfoliados junto al ápice; lodículas 2, de 0.3 mm de largo, conduplicadas, truncadas; estambres 3, anteras de 1.5 mm de largo. *Cariopsis* largamente elipsoide, de 1.8 mm de largo, 0.7 mm de ancho, blanquecino; hilo oblongo; embrión ⅓ del largo de la cariopsis.

Distribución y ecología. Conocida sólo para Ecuador, habita bajo matorrales en bordes de caminos a 2430 m.

Especie afín a *Paspalum reclinatum* de la que se distingue por, tener espiguillas con la gluma superior fuertemente corrugada a lo largo de los nervios marginales, raquis de las ramificaciones estrecho, de 0.5–0.8 mm de ancho, y antecio superior cartilaginoso, con tintes violáceos a totalmente violáceo a la madurez.

9. **Paspalum sodiroanum** Hack., Oesterr. Bot. Z. 51: 237. 1901. *Paspalum sodiroanum* Hack. in Sodiro, Anales Univ. Centr. Ecuador 1889: 3. 1889, nom. nud. TIPO: Ecuador. Sin localidad, 2000 m, 1886, *Sodiro s.n.*** (holótipo, W no visto, fragmento, US-285600; isótipo, P). Figuras 9, 14.

Paspalum sodiroanum Hack. var. *terminale* Hack. in Sodiro, Anales Centr. Ecuador 1889: 4. 1889, nom. nud.

Paspalum peruvianum Mez, Repert. Spec. Nov. Regni Veg. 15: 28. 1917. TIPO: Perú. Lima: Huarochirí, entre Tambo de Viso y Chaupichaca, 2650–2880 m, *Weberbauer 137* (holótipo, B, fragmento, y foto, US-2942537).

Plantas anuales, con *cañas* de (20–)35–100 cm de alto, decumbentes a geniculadas, arraigadas y ramificadas en los nudos inferiores, glabras; entrenudos de 6–16 cm de largo, surcados, pajizos a morados; nudos glabros, castaños. *Vainas* de 4–10 cm de largo, estriadas, glabras o con escasos pelos sobre los márgenes apicales. *Lígulas* de 1–2.4 mm de largo, hialinas, de ápice truncado, castañas; cuello glabro. *Láminas* lanceoladas a linear-lanceoladas, de 3–17 cm de largo, 0.5–1.5 cm de ancho, delgadas, glabras a esparcidamente pilosas, de base redondeada a subcordada, no pseudopecioladas, el ápice acuminado, los márgenes cartilaginosos, blanquecinos, escabrosos, en ocasiones ciliados hacia la base. *Pedúnculos* hasta de 20 cm de largo, angulosos, estriados, glabros. *Inflorescencias* de contorno largamente ovado, de (4–)6–16 cm de largo, 2–6 cm de ancho; eje principal anguloso, surcado, glabro; pulvínulos largamente pilosos, con pelos blanquecinos hasta de 2 mm de largo; ramificaciones (7–)15–35, alternas a subopuestas, rectas; raquis de las ramificaciones de 2–4 cm de largo, 2–3 mm de ancho hacia la base, de ápice atenuado y terminando en una espiguilla o en una prolongación estéril, la cara adaxial glabra o con pelos esparcidos sobre el nervio medio, cara abaxial cortamente pubescente, los márgenes cartilaginosos, blanquecinos; pedicelos de 0.5 mm de largo, aplanados. *Espiguillas* elipsoides, de 2.1–

FIGURA 14. *Paspalum sodiroanum* Hack. (A de *Acosta Solís 17451*, B H de *Pohl et al. 15440*).—A. Hábito.—B. Espiguilla vista del lado de la gluma superior.—C. Espiguilla vista del lado de la lemma inferior.—D. Gluma superior. E. Lemma inferior.—F. Lemma inferior encerrando al antecio superior.—G. Antecio superior visto del lado de la lemma superior.—H. Antecio superior visto del lado de la pálea superior.

2.7 mm de largo, 1–1.2 mm de ancho, imbricadas, biseriadas, abiertas a la madurez. *Gluma superior* y *lemma inferior* subiguales, tan largas como la espiguilla, lisas, glabras, delgadas, pálidas o con tintes rojizos a púrpuras, 0.5–0.7 mm más largas que el antecio superior, 3-nervias. *Antecio superior* elipsoide, de 1.8–2.4 mm de largo, 0.8–1 mm de ancho, 0.5–0.7 mm más corto que la espiguilla, caedizo, crustáceo, blanco brillante; lemma y pálea con papilas simples, más marcadas sobre la pálea, y micropelos bicelulares en toda la superficie; lodículas 2, de 0.4 mm de largo, abrazando los bordes inferiores de la pálea superior; estambres 3, anteras de 1.2 mm de largo, castañas. *Cariopsis* elipsoide, de 1.3–1.6 mm de largo, 0.6–0.7 mm de ancho; hilo oblongo; embrión ⅓ del largo de la cariopsis.

Nombre vulgar. "Grama," "nudillo," "pasto negro," "maicillo," "siquiquigua" (Ecuador).

Distribución y ecología. Colombia, Ecuador, y Perú, hallándose en suelos arcillosos, siendo frecuente en campos cultivados; es palatable para el ganado. Crece entre 1500 y 2850 m.

Material adicional examinado. COLOMBIA. **Antioquia:** Yarumal, *Carbonó 1779** (UTMC); Marinilla, entre Marinilla y El Peñol, 2100 m, 28 Ago. 1986, *Pohl et al. 15440* (MO). **Caldas:** Finca La Selva, vía Arauca, 1500 m, *Gutiérrez et al. 33* (COL). **Caquetá:** Florencia, Hacienda Corinto, Mayo 1940, *Plata 75* (US). **Cauca:** Popayán, 1500–2000 m, *Lehmann 5938, 6342* (US); Chisquío, finca Los Derrumbos, ca. 1700 m, 3 Feb. 1940, *Asplund 10479* (US). **Cundinamarca:** San Francisco, 2000 m, 11 Abr. 1988, *Franco et al. 2482** (COL). **Nariño:** Chachgüí, 1500–2000 m, Jun. 1966, *Martínez G. & Martínez 6** (COL). **Putumayo:** Valle de Sibundoy, ca. 2200 m, 5 km S of Sibundoy, 10 Feb. 1963, *Chindoy 83* (COL, US); Valle de Sibundoy, ca. 2200 m, 3 km S de Sibundoy, 17 Mar. 1963, *Bristol 641** (COL). **Sin Departamento:** sin localidad, *André s.n.* (P, US). ECUADOR. **Imbabura:** El Porvenir, Río Chamachán, áreas de Buenos Aires, 2700 m, 4 Jul. 1950, *Acosta Solís 17451* (US); Natabuela, 2410 m, 21 Ago. 1950, *Acosta Solís 17947* (US); Partidero de Chota a Juncal, 1500–1600 m, 30 Dic. 1949, *Acosta Solís 14990* (US); Valle de Chota, 1600 m, 29 Dic. 1949, *Acosta Solís 14905* (US); Pimampiro, 2050 m, 21 Nov. 1949, *Acosta Solís 14570* (US). **Pichincha:** Puíllaro, 2100 m, 16 Abr. 1950, *Acosta Solís 16462* (US); Parroquia Calacali, Reserva Geobotánica Pululahua, 00°05′N, 78°30′W, 1800–3356 m, 8 Ago. 1987, *Cerón et al. 1844* (MO). **Sin Departamento:** Niebly, *Sodiro s.n.* (US).

Especie afín a *Paspalum flavum*, de la que se separa por poseer racimos de 2–3 mm de ancho, espiguillas de 2.1–2.7 mm de largo, no papilosas, y antecio superior de 1.8–2.4 mm de largo.

10. Paspalum soukupii Carbonó, sp. nov. TIPO: Perú. Lima: San Bartolomé, Quebrada Verrugas, ca. 2000 m, 8 Ago. 1964, *Soukup 5282**,** (holótipo, US-2528419). Figuras 9, 15.

Haec species *Paspalo candido* (Humb. & Bonpl. ex Flüggé) Kunth similis sed lemma inferna nulla differt.

Plantas anuales, cespitosas, con *cañas* erectas, ramificadas en los nudos inferiores, de 30–50 cm de alto; entrenudos de 5–13 cm de largo, glabros, huecos, estriados, pajizos, los entrenudos superiores más largos que las vainas; nudos largamente pilosos, con pelos blanquecinos a glabros, castaños. *Vainas* de 4–10 cm de largo, esparcidamente pilosas con pelos más abundantes hacia el ápice, los márgenes pilosos. *Lígulas* de 1–1.5 mm de largo, membranáceas, hialinas; cuello cortamente piloso. *Láminas* linear-lanceoladas, de 3–9 cm de largo, 0.3–0.6 cm de ancho, delgadas, las superiores más reducidas, de base redondeada, no pseudopecioladas, y ápice acuminado, esparcidamente pilosas en ambas caras, los márgenes escabrosos. *Pedúnculos* hasta de 17 cm de largo, cilíndricos, glabros, lisos. *Inflorescencias* exertas o subincluidas en las vainas superiores, de contorno elíptico, de 4–8 cm de largo, 1.5–4 cm de ancho; eje principal glabro o esparcidamente piloso, anguloso, surcado; pulvínulos cortamente pilosos; ramificaciones 8–23, ascendentes, alternas a subopuestas; raquis de las ramificaciones de 1–3.3 cm de largo, 1.8–2.5 mm de ancho, con cara adaxial glabra, la cara abaxial cortamente pilosa, los márgenes lisos, escabriúsculos hacia la porción distal, ápice del raquis terminando en una prolongación estéril; pedicelos de 0.3–0.5 mm de largo, aplanados. *Espiguillas* ovoides a elipsoides, de 2–2.7 mm de largo, 0.7–0.9 mm de ancho, uniseriadas, de ápice agudo. *Gluma superior* y *lemma inferior* ausentes. *Antecio superior* tan largo como la espiguilla, blanco a violáceo, cartilaginoso, brillante; lemma con papilas simples en toda su superficie y cuerpos de sílice exfoliados hacia el ápice; pálea con papilas simples en toda su superficie, micropelos bicelulares hacia los bordes junto con la lemma y cuerpos de sílice exfoliados hacia el ápice; estambres 3, anteras de 1.4–1.7 mm de largo. *Cariopsis* elipsoide, de 1.7 mm de largo, 0.8 mm de ancho, castaño; hilo oblongo; embrión ⅓ del largo de la cariopsis.

Distribución y ecología. Perú, en laderas rocosas a orillas de quebradas y ríos, entre los 2000 y 2600 m.

Material adicional examinado. PERU. **Lima:** 6 km above Matucana, canyon of Río Rimac, just above first tunnel and before bridge, 2560–2600 m, 19 Abr. 1964, *Hutchinson & Ricardi 4997**,** (MO, US).

Especie afín a *Paspalum candidum*, de la cual

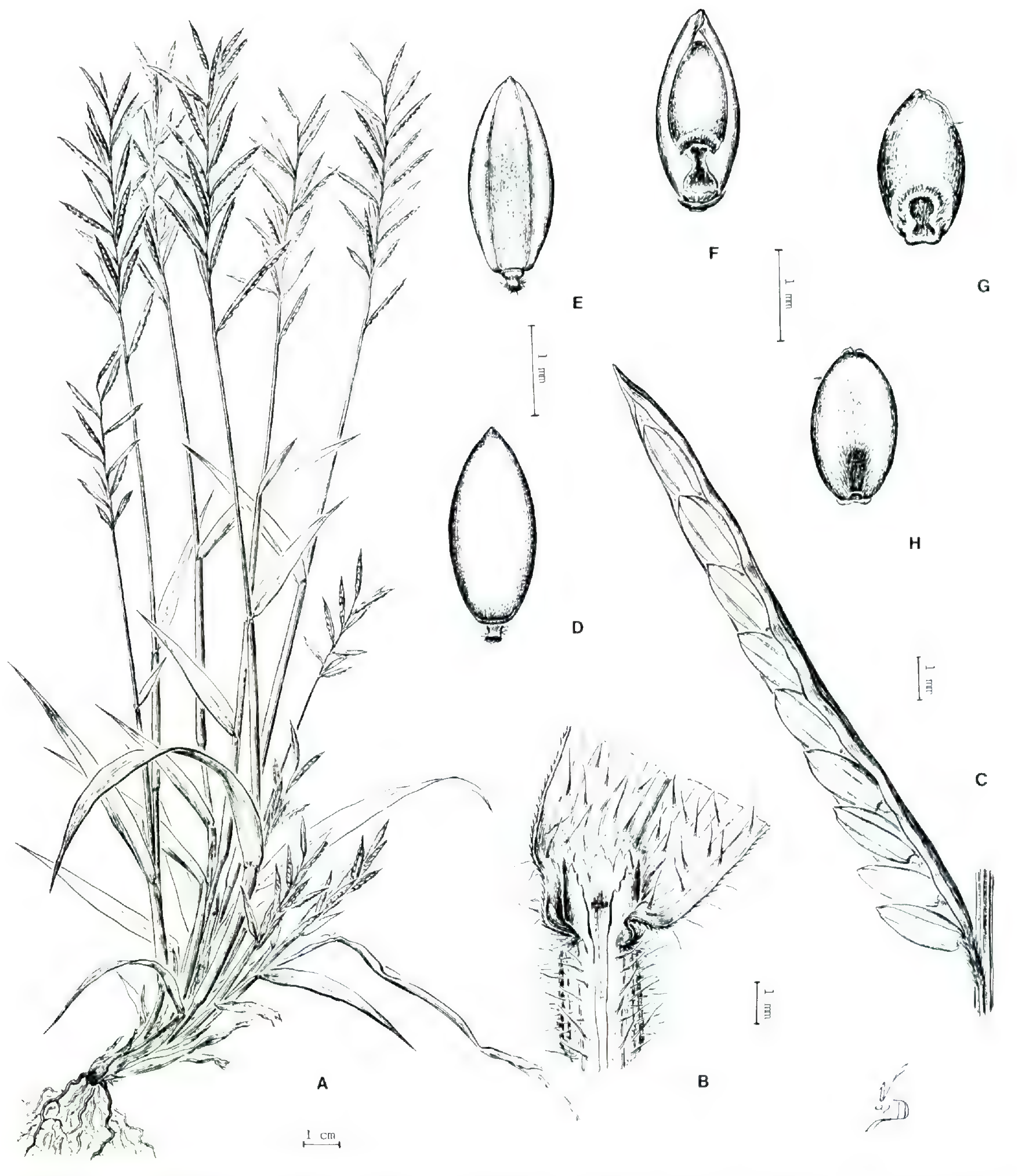


FIGURA 15. *Paspalum soukupii* Carbonó (de *Hutchinson & Ricardi 4997*).—A. Hábito.—B. Detalle de la región ligular.—C. Racimo de la inflorescencia.—D. Antecio superior visto del lado de la lemma superior.—E. Antecio superior visto del lado de la pálea superior.—F. Pálea superior encerrando el cariopsis.—G. Cariopsis, vista escutelar.—H. Cariopsis, vista hilar.

se distingue por la ausencia de la lemma inferior.

Tovar (1993) al tratar las gramíneas del Perú incluye al espécimen *Soukup 5282* en el material estudiado de *P. depauperatum* (= *P. candidum*).

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11847 (1), 12196 (1), 12715 (6). Salaverry 122 (5). Sánchez 76 (1), 2995 (6). Sánchez Vega 3452 (1). Savatier *s.n.* (2), *s.n.* (6), 1188 (6). Schimpff 518 (6). Schreiter 4473 (5). Schulz 99 (5), 658 (5), 658a (5), 743 (5). Seibert 2227 (1), 2293 (1), 2299 (1). Seidel 997 (1). Simpson 463 (1). Sin colector, ex SI 16452 (5). Skutch 3397 (1). Smith 1762 (1), 2180 (1), 4992 (1), 10901 (1), 12160 (5). Sneidern 157 (1), 1287 (1), 2112 (3). Sodiro *s.n.* (6), *s.n.* (8), 1887 (1). Solomon 3190A (1), 3193 (3), 13377 (5), 13692 (3), 14340 (3), 16379 (1), 16513 (5), 16671 (1), 18263 (5), 18498 (3). Soukup 1845 (2). Sparre 14619 (3). Spiaggi 23 (3). Spruce 5943 (3), 5954 (6), 5960 (1). Standley 19787 (1), 32268 (1), 32779 (1), 39016 (1), 41256 (1), 41606 (1), 43214 (1), 47838 (1), 50018 (1), 50626 (1), 58967 (1), 60806 (1), 77431 (1), 81035 (1), 90151 (1). Steinbach 9579 (5). Stewart 1320 (3). Steyermark 118655 (4). Stordy *s.n.* (5). Stork 9344 (2). Sullivan 844 (2), 967 (2). Swallen 10908 (1). Sydow 449 (1).

Tamayo 1859 (3), 2274 (5), 2313 (4). Taylor 17437 (1). Toledo T-2867 (1). Tonduz 9854 (1). Toro 675 (1). Torres 14 (1). Tovar 375 (3), 1905 (1), 2040 (1), 2164 (5), 2983 (5), 3135 (5), 3614 (3), 3778 (1), 4065 (1), 5301 (5), 5345 (1). Triana *s.n.* (6), 258 (1), 259 (1), 764 (4). Tupayachi 182 (3), 228 (1). Türckheim 3970 (1). Türpe 1156 (5).

van der Werff 7673 (1), 7001 (5). Valencia 17 (4). Vareschi 5775 (5). Vargas 285 (5), 1201 (2), 1005 (1), 1862 (3), 2158 (1), 3310 (5), 3575 (1), 5697 (5), 7156 (5), 7206 (3), 7673 (3), 11165 (5), 11206 (5), 11574 (5), 17414 (1). Velarde Núñez 2244 (3), 2619 (1), 5011 (2). Venturi 3025 (5), 3041 (5), 8392 (5). Villavicencio 288 (5), 525 (5), 810 (5), 819 (5).

Warmings *s.n.* (1). Weatherwax 165 (1). Weaver 921 (1). Weberbauer 415 (5), 5707 (2). Weddell 4423 (5), 4490 (5). Wilbur 13525 (1). Williams 13613 (1), 17305 (1), 20084 (1), 23404 (1), 25967 (1), 26932 (1), 42841 (1). Wood 3569 (1), 4305 (7), 4357 (1). Wurdack 492 (1), 572 (1), 713 (1), 811 (1).

Yepes-Agredo 3132 (4).

Zapata *s.n.* (6). Zuloaga 4259 (7).

TAXONOMIC REVISION OF THE ANT-ACACIAS (FABACEAE, MIMOSOIDEAE, *ACACIA*, SERIES GUMMIFERAE) OF THE NEW WORLD[1]

David S. Seigler[2] *and*
John E. Ebinger[3]

Abstract

Detailed descriptions, habitat preferences, geographic ranges, and representative specimens are given for the 13 ant-acacia species and hybrids from Mexico, Central America, and South America. A principal component analysis (PCA) of vegetative and floral features shows these species form discrete groups in plots of the first three principal components. Taxa were found to be relatively homogeneous, as indicated by the tight clusters formed in the PCA plots. The data suggest that there is very little gene flow between species, and hybrids are extremely uncommon. Of the 13 species in this group, *Acacia ruddiae* is only marginally a myrmecophyte, rarely being inhabited by acacia-ants, and apparently never producing Beltian bodies on the tips of the leaflets. The remaining taxa have stipular spines that usually are inhabited by obligate acacia-ants of the genus *Pseudomyrmex*, produce Beltian bodies, and have enlarged petiole and rachis glands.

There are about 50 species of the genus *Acacia* Miller series *Gummiferae* Benth. (Fabaceae: Mimosoideae) in the New World. The taxa of this series are quite distinct and are diverse in habitat, habit, morphology, and chemistry. Most authors agree that the series *Gummiferae* is a natural, monophyletic unit composed of trees and shrubs that lack prickles but have stipular spines, bipinnately compound leaves, globose heads or cylindrical spikes that are solitary or borne in axillary fascicles, and legumes that are dehiscent or indehiscent, mostly with uniseriate seeds (Bentham, 1875; Pedley, 1978; Vassal, 1969–1972). Britton & Rose (1928), however, subdivided this series into a number of genera. Most of these "genera" currently are not accepted, but they often circumscribe groups of species at the level of subgenus or section. *Tauroceras* and *Myrmecodendron* are two such segregates proposed by Britton & Rose (1928) that are separated from other *Acacia* species by various fruit characteristics, the involucre of the flowering peduncle, and the large, ant-inhabited stipular spines.

The 13 ant-acacias are restricted to relatively dry to wet, lowland habitats from the coastal regions of central Mexico to northwestern Colombia. This group of neotropical myrmecophytes shares many adaptive ecological and morphological traits, most of which appear to be related to their mutualistic association with acacia-ants of the genus *Pseudomyrmex* (Janzen, 1966, 1974). All have: enlarged stipular spines that are usually inhabited by ants (Fig. 1A, B, C); Beltian bodies, which are used as a food source by the ant larvae, on the tip of many leaflets (Fig. 1D); and enlarged petiolar glands (Fig. 1E, F, G). Many have rachis glands that continuously secrete nectar, many produce new leaves throughout the year, and some have lost chemical and structural traits that protect other *Acacia* species from herbivores (Rehr et al., 1973).

For the most part, ant-acacia species are distinct units that are clearly delimited by a number of morphological traits. Furthermore, in areas where two or more species grow side by side, intermediate plants seldom are encountered (Janzen, 1974), indicating that these species hybridize only rarely with each other (Ebinger & Seigler, 1992).

Materials and Methods

More than 2000 specimens from 14 herbaria (A, CM, EIU, F, GH, ILL, LL, MEX, MINN, MO,

[1] We thank the curators of the many herbaria which sent plant material for study and acknowledge support by the National Science Foundation (NSF BSR 82-15274) and the U.S. Department of Agriculture OICD research agreement (58-319R-0-011), and a grant from the American Philosophical Society.
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[3] Botany Department, Eastern Illinois University, Charleston, Illinois 61920, U.S.A.

Ann. Missouri Bot. Gard. 82: 117–138. 1995.

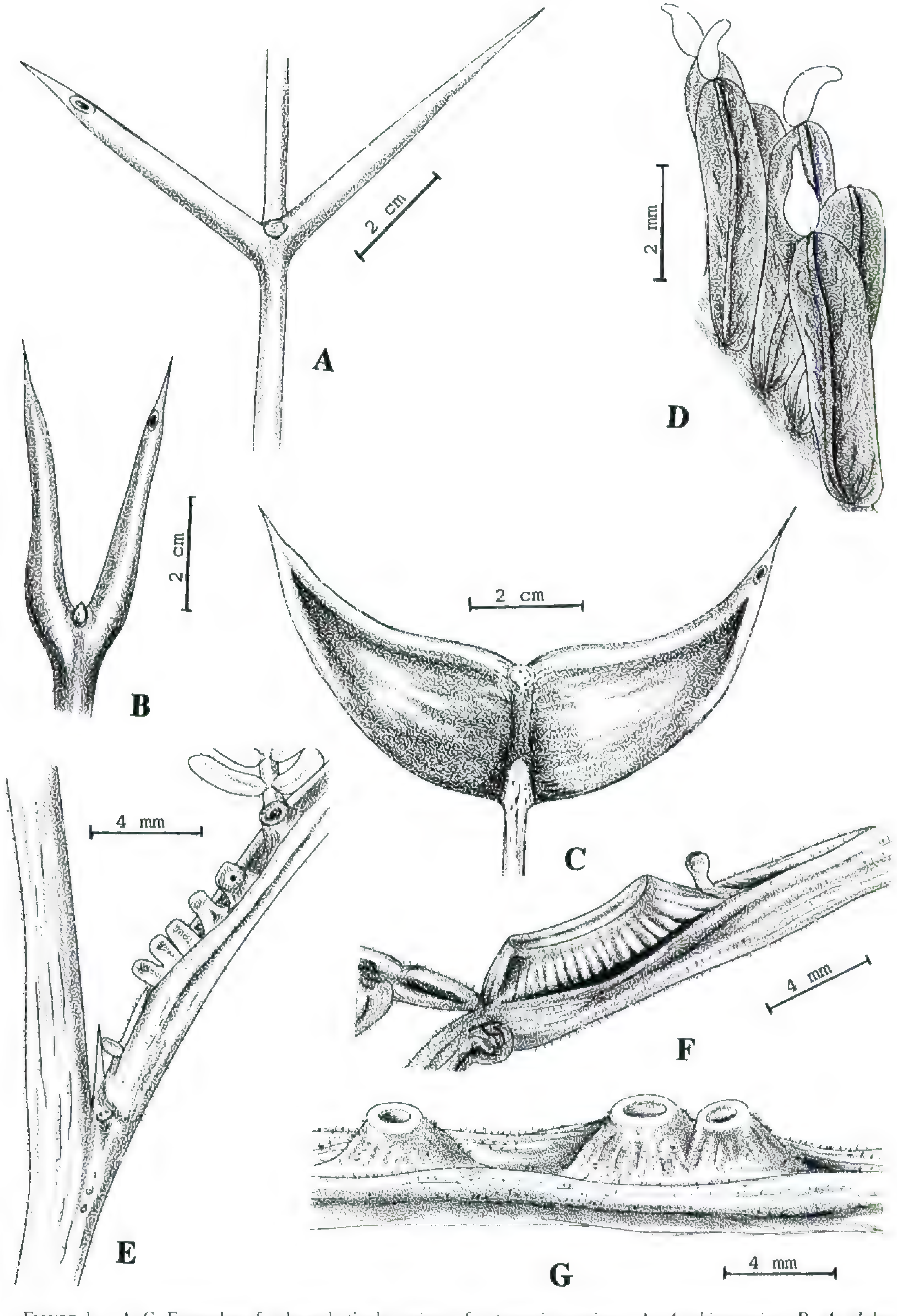

FIGURE 1. A–C, Examples of enlarged stipular spines of ant-acacia species.—A. *A. chiapensis.*—B. *A. globulifera.*—C. *A. hindsii.*—D. Beltian body on a leaflet of *A. chiapensis.* E–G, Examples of petiole glands of some ant-acacia species.—E. *A. chiapensis.*—F. *A. cornigera.*—G. *A. globulifera.*

TABLE 1. Characters scored for the principal component analysis of the 13 species of ant-acacias.

1. Stipular spines inhabited by ants: 1 = not inhabited, 2 = inhabited.
2. Stipular spine pubescence: 1 = glabrous to lightly puberulent, 2 = densely puberulent, 3 = pubescent with yellowish hairs.
3. Stipular spine surface: 1 = smooth, 2 = longitudinal ridges and grooves, 3 = longitudinal flanges.
4. Stipular spine symmetry: 1 = symmetrical, 2 = asymmetrical.
5. Leaf length: 1 = most less than 200 mm long, 2 = most more than 200 mm long.
6. Average number of pairs of pinnae per leaf.
7. Rachis glands: 1 = absent, 2 = present.
8. Petiolar glands: 1 = one present, 2 = two or more present, 3 = absent.
9. Petiolar and rachis gland shape: 1 = volcano- to columnar-shaped, 2 = narrow and elongated to canoe-shaped.
10. Beltian bodies: 1 = absent, 2 = present.
11. Leaflet length: 1 = less than 5.9 mm long, 2 = more than 6 mm long.
12. Secondary veins of leaflets: 1 = not obvious, 2 = obvious.
13. Thickness of flowering peduncle: 1 = less than 1.9 mm thick, 2 = more than 2 mm thick.
14. Inflorescences per node: 1 = 1–8, 2 = 9 or more.
15. Inflorescence shape: 1 = globose, 2 = cylindrical.
16. Floral bract apex shape: 1 = circular, 2 = tailed.
17. Calyx/corolla ratio: 1 = calyx about half the length of the corolla, 2 = calyx nearly as long as the corolla.

NY, TEX, UC, and US) were studied to determine the geographic range and morphological variation of the ant-acacia taxa. These specimens were sorted into groups based on similarity of morphological characteristics. Virtually all of the specimens could be placed in groups corresponding to the taxa listed below; only a few intermediate individuals were found (Ebinger & Seigler, 1992). Ninety-three specimens representing the geographical and morphological range of each taxon were scored for 12 vegetative and five floral characters (Table 1). All characters were measured (three or more measurements from each specimen) and plotted to confirm that gaps existed in order to enable the use of scored characters. These differences were observed in all cases. The data were then analyzed by principal components analysis (PCA) with the BMDP Statistical Software Package (Dixon, 1983) and by NTSYS-pc (Rohlf, 1990). Groups were analyzed separately and in various combinations, as were apparently hybrid specimens and their putative parental taxa. Subsequently all taxa were analyzed simultaneously.

All specimens used in this study were tested for the presence of cyanogenic compounds by the Feigl-Anger method (Feigl & Anger, 1966; Tantisewie et al., 1969; Brinker & Seigler, 1989). A few leaflets from each specimen were crushed, placed in a small vial, and moistened with distilled water. A strip of filter paper impregnated with copper ethylacetoacetate and tetra base (4,4′-tetramethyldiaminodiphenylmethane) was added to the vial so as not to touch the sample, and the vial sealed with a cork. The presence of cyanide was indicated if the filter paper turned a deep blue color.

RESULTS AND DISCUSSION

When the entire set of 93 specimens was analyzed, the first three principal components accounted for 38%, 17%, and 12% respectively, or 67% of the total variance. The amount of variance contributed by the remaining components diminished slowly from the third principal component onward. Plots of all pairs of the first three principal components indicated that the only phenetic groupings were those found in the ordination based on the first two components.

A plot of the first two principal components revealed 13 distinct groups corresponding to the previously defined species (Fig. 2). Calyx/corolla length ratio, inflorescence shape, and the presence or absence of rachis glands (characters 17, 15, and 7) were the most important characters for determining the component score of the first principal component. Stipular spine symmetry, number of inflorescences per node, and stipular spine pubescence (characters 4, 14, and 2) were the most important in determining the second principal component (Table 1).

Distinct OTUs can be recognized in the PCA plot (Fig. 2). The clusters do not seem to contain recognizable subgroups, and each cluster is separated from the others. Also, the points for specimens in each group are closely spaced, indicating that the species are homogeneous. Similar results were obtained when the entire complex was divided into smaller species groups and analyzed by PCA.

TAXONOMIC TREATMENT

The present systematic treatment is based on herbarium specimens as well as extensive collections and observations of these taxa in Mexico and Central America by the first author (DSS) on 13 trips during 1975–1993. Only important diagnostic characteristics were used to distinguish the taxa, and only enough representative specimens to indicate the distribution of each species are included. Most specimens used in the PCA analysis also are included in the list of representative specimens.

FIGURE 2. Plot of axis 1 vs. 2 for principal component analysis, using 12 vegetative and five floral variables, of 93 specimens of ant-acacia species.

For most accepted names and synonyms, the type specimens are readily available. For some, however, particularly for names proposed by Schenck (1913) and Schlechtendal & Chamisso (1830), the types, which were probably located at B, have been destroyed (H. Ern, pers. comm.). For these species, lectotypes have been designated.

The ant-acacias are closely related to the *Acacia macracantha* Humb. & Bonpl. ex Willd, *A. cochliacantha* Humb. & Bonpl. ex Willd., and *A. pennatula* (Schltdl. & Cham.) Benth. group in the series *Gummiferae*. In addition to the myrmecophyte characteristics of enlarged stipular spines, enlarged petiolar glands, and the usual production of Beltian bodies, the ant-acacias are shrubs or small or medium-sized trees with bipinnately compound leaves that bear one or more extrafloral nectaries on the petiole and/or rachis. The inflorescences are spicate or capitate and fascicled or solitary in the axils of the leaves, or sometimes on leafless shoots. The flowers are small (2–5 mm long) and subtended by a small, peltate bract. The sepals and petals are connate and 4–5-merous. The stamens are numerous (50 or more) and exserted. There is a single carpel which develops into a dehiscent or indehiscent legume.

KEY TO THE SPECIES OF ANT ACACIAS

1a. Petiolar glands usually saddle-shaped or canoe-shaped, or long and narrow, lower than the petiolar groove and nearly as long as the petiole.

2a. Petiolar glands long and narrow, lower than the petiolar grooves and nearly as long as the petiole .... 4. *A. cookii*

2b. Petiolar glands saddle-shaped or canoe-shaped.
  3a. Spines with two longitudinal flanges; leaflets more than 10 mm (usually 12–18 mm) long .... 10. *A. mayana*
  3b. Spines lacking longitudinal flanges; leaflets less than 10 mm long.
    4a. Leaflets with 2–3 veins from the base, lateral veins obvious; inflorescence more than 3 times longer than wide .... 5. *A. cornigera*
    4b. Leaflets with one vein from the base, lateral veins not obvious; inflorescence subglobose, less than 2 times longer than wide .... 13. *A. sphaerocephala*

1b. Petiolar glands dome-shaped, volcano-shaped, or columnar, rarely absent.
  5a. Inflorescence cylindrical, more than 5 times longer than wide.
    6a. Leaflets with one vein from the base, lateral veins not obvious; enlarged stipular spines flattened at the base .... 8. *A. hindsii*
    6b. Leaflets with 2–5 veins from the base, lateral veins obvious; enlarged stipular spines terete or oval in cross section.
      7a. Petiolar glands usually solitary; rachis glands scattered along the leaf rachis .... 6. *A. gentlei*
      7b. Petiolar glands 3–5; rachis glands absent .... 3. *A. collinsii*
  5b. Inflorescence globose, never more than 2 times longer than wide.
    8a. Leaflets more than 1.8 mm wide, with 2–3 veins from the base, lateral veins obvious .... 1. *A. allenii*
    8b. Leaflets mostly less than 1.8 mm wide, with one vein from the base, lateral veins not obvious.
      9a. Adaxial surface of the petiole flattened (on well-developed leaves) with numerous petiolar glands (6–30) present on the flattened surface .... 11. *A. melanoceras*
      9b. Adaxial surface of the petiole grooved with fewer than 13 petiolar glands present in the groove.
        10a. Stipular spines asymmetrical, commonly curved around the stem, pubescent with yellowish hairs .... 9. *A. janzenii*
        10b. Stipular spines symmetrical, glabrous to puberulent.
          11a. Rachis glands cylindrical and narrowing toward the base; most stipular spines not enlarged, those that are usually not inhabited by acacia-ants .... 12. *A. ruddiae*
          11b. Rachis glands narrowly volcano-shaped, or columnar and not narrowing toward the base; most stipular spines enlarged and inhabited by acacia-ants.
            12a. Petiolar and rachis glands columnar; inflorescences in clusters of 10–40; fruit margins ridged .... 2. *A. chiapensis*
            12b. Petiolar and rachis glands narrowly volcano-shaped; inflorescences in clusters of 4–8; fruit margins not ridged .... 7. *A. globulifera*

1. Acacia allenii D. H. Janzen, Smithsonian Contr. Bot. 13: 53. 1974. TYPE: Costa Rica. Puntarenas: Osa Peninsula, bank of tributary of Río Agua Buena, 3.5 mi. SW of Rincón, 9 Mar. 1967, *D. H. Janzen 1769* (holotype, US; isotypes, F, GH, K, MEXU, MICH, MO, NY, UC).

Tree to 25 m tall with widely spreading branches, young twigs dark brown to dark reddish brown, lightly puberulent. Stipular spines black to dark brown, commonly grooved and with low, rounded longitudinal ridges, glabrous to lightly puberulent, nearly terete in cross section, sometimes slightly reflexed, symmetrical, broadly V-shaped with an angle of 90–120°, to 45 mm long, 8–12 mm wide near the base. Leaves 150–400 mm long, pinnae 10–22 pairs per leaf, 60–110 mm long, 15–22 mm between pinna pairs; rachis grooved, lightly puberulent, rachis glands puberulent, lightly striate, narrowly volcano-shaped, 1 or 2 located at the node of each pinna pair, sometimes scattered along the rachis between the pinna pairs; petiole grooved, lightly puberulent, 14–30 mm long. Petiolar glands narrowly volcano-shaped, usually 4 10, scattered along the petiole, puberulent, lightly striate, apex 0.4–0.7 mm across, base 0.8–1.2 mm across. Leaflets 15–25 pairs per pinna, glabrous, linear, 6–12 mm long, 1.8–3.9 mm wide, lateral veins obvious, 2–3 veins from the base, apex obtuse to rarely acute. Inflorescence a densely flowered globose head, 5–7 mm across, in axillary clusters of 5–15 in the axil of reduced leaves, commonly on short axillary branches 40–150 mm long; peduncles lightly puberulent, 10–15 mm long, 0.6–0.9 mm thick, nearly the same thickness throughout; involucre located on the lower ⅓ of the peduncle, puberulent, 5-lobed. Floral bracts peltate, apex circular, ciliate, stalk 0.8–1.0 mm long. Flowers sessile; calyx 5-lobed, puberulent on the lobes, 0.9–1.2 mm long; corolla 5-lobed, glabrous, pale yellow, 1.2–1.5 mm long. Legumes (only one specimen, *Janzen 11051*, with fruits seen) slightly curved, flattened, 65–110 mm long, 15–20 mm wide, glabrous, longitudinally striate, dark brown to black, dehiscent along two sutures, base not stipitate, the apex acute. Flowering April–July.

Distribution. Heavily shaded understory at lower elevations (to 160 m) in wet forests, in moist disturbed stream banks, at forest edge, and on roadsides, in Puntarenas Province, Costa Rica.

Representative specimens. COSTA RICA. **Puntarenas:** forested hills near Golfito de Golfo Dulce, *Allen 5997* (F, GH, MO, US); 5 mi. SW of Rincón, Osa Peninsula, *Janzen 791* (F, GH, MEX, MO).

The narrow geographic range of *Acacia allenii* is typical of many of the ant-acacia species of Central America. Its normal habitat appears to be at lower elevations in heavily shaded understory of wet tropical forests. In general, it reacts fairly well to disturbances, and most present-day collections are from roadsides, recent landslides, disturbed stream banks, and secondary successional areas after logging. It is rarely found in open pastures, and according to Janzen (1974) will not survive fire.

As is typical of many wet forest ant-acacias, Beltian body production in *Acacia allenii* is not particularly extensive. However, the Beltian bodies are much larger (to 3 mm long) than those found in dry area ant-acacias. In *A. allenii*, these relatively large Beltian bodies are formed only on the lower 3–6 leaflets of each pinna, and occasionally the lower leaflet of a pinna has been replaced by a large Beltian body. None of the individuals tested positive for cyanide production.

Acacia allenii is most closely related to *A. melanoceras* and possibly *A. collinsii.* The globose inflorescences and the presence of rachis glands easily separate this species from *A. collinsii*, which has elongated inflorescences and usually lacks rachis glands. It is easily separated from *A. melanoceras*, since its leaflets are nearly twice as large and have obvious secondary veins. Also, well-developed leaves of *A. allenii* have only 4–10 widely scattered petiolar glands, while *A. melanoceras* commonly has 6–30 closely spaced petiolar glands on the flattened adaxial surface of the petiole. Furthermore, the fertile branches of *A. allenii* are commonly less than 150 mm long, are scattered along major branches throughout the crown, and have 5–15 inflorescences in the axis of reduced leaves. In *A. melanoceras*, the fertile branches are to 350 mm long, tend to be bunched near the ends of the sterile branches, and have 2–6 inflorescences at each leafless node.

2. **Acacia chiapensis** Safford, J. Wash. Acad. Sci. 5: 356. 1915. TYPE: Mexico. Chiapas: near San Fernando, between Tuxtla and Chicoasén, 12 Jan. 1907, *G. N. Collins 164* (holotype, US; photo, F).

Shrub or small tree to 10 m tall, young twigs brown to reddish brown, usually densely puberulent. Stipular spines (Fig. 1A) usually dark brown to black, smooth, terete, usually densely puberulent (rarely glabrous), symmetrical, V-shaped with an angle of 70–100°, straight to slightly reflexed near the apex, 30–80 mm long, 4–7 mm thick near the base. Leaves 100–250 mm long; pinnae 12–30 pairs per leaf, 30–70 mm long, 5–12 mm between pinna pairs; rachis grooved, densely puberulent, a columnar gland commonly present between each pinna pair (sometimes absent); petiole grooved, densely puberulent, 7–17 mm long. Petiolar glands (Fig. 1E) columnar, densely puberulent, striate, apex 0.5–0.8 mm across, base 0.6–1.2 mm across, usually 6–13 scattered along the petiole. Leaflets 22–50 pairs per pinna, glabrous, linear, 3–8 mm long, 0.7–1.6 mm wide, lateral veins not obvious, only one vein from the base, apex acute to obtuse. Inflorescence a densely flowered globose head 5–9 mm across, in clusters of 10–40 in the axil of slightly reduced leaves, or in clusters of 10–40 in the axil of much reduced leaves on axillary branches; peduncles glabrous to lightly puberulent, 10–35 mm long, 0.6–1.1 mm thick, nearly the same thickness throughout; involucre located near the middle of the peduncle, glabrous to puberulent, 4-lobed. Floral bracts peltate, apex circular, stalk 0.8–1.5 mm long. Flowers sessile; calyx 5-lobed, glabrous, 1.0–1.3 mm long; corolla 5-lobed, glabrous, pale yellow, 1.6–1.9 mm long. Legume nearly straight, flattened, 65–90 mm long, 8–10 mm wide, glabrous, longitudinally striate, black to dark brown, tardily dehiscent, stipe less than 5 mm long, the apex narrowing to a very short spinelike beak, margins ridged. Flowering January–May.

Distribution. Usually rare in dry lowland sites, and along and near watercourses in heavily disturbed vegetation in extreme southern Mexico.

Representative specimens. MEXICO. **Chiapas:** steep slope with montane rainforest, 18–20 km N of Ocozocoautla along road to Mal Paso, 800 m, *Breedlove 23862* (MEX, MICH, MO, NY, TEX). **Oaxaca:** gravel pit, second growth vegetation, 1 mi. W of Temascal, *Janzen 185* (F, MEX, MO). **Veracruz:** Dos Ríos, *Mell 506* (NY).

Acacia chiapensis appears to be most closely related to *A. globulifera* in its morphology and habit. These two species are easily separated, because in *A. chiapensis* there are usually 6–13 columnar petiolar glands scattered along the petiole, the globose inflorescences are in axillary clusters of 10–40, and the legume has a distinct marginal ridge. *Acacia globulifera*, in contrast, usually has only 3–6 narrow, volcano-shaped petiolar glands on a petiole, the globose inflorescences rarely exceed 8 in a cluster, and the legume lacks marginal ridges. The only other species with which *A. chia-*

pensis could be sometimes confused is *A. collinsii*, but this species has elongated inflorescences, fewer petiolar glands, and its leaflets have obvious secondary veins.

Acacia chiapensis is usually shrubby, but can reach a height of 10 m (Janzen, 1967b). When repeatedly cut it commonly forms a dense bush, but rarely produces root sprouts. In many respects this species has a life form similar to that of non-ant-acacias such as *A. farnesiana* and *A. macracantha*. It is similar to these species in seed dispersal, seedling ecology, low Beltian body production, and the fact that many leaves on elongated lateral branches are not subtended by swollen spines (Janzen, 1974).

It appears that *A. chiapensis* is a marginal host for obligate acacia-ants. Janzen (1974) indicated that this species can sometimes survive in the absence of ants. Most specimens of this species retain cyanogenic glycosides in the leaves, which probably limits herbivory (Seigler & Ebinger, 1987). Low Beltian body production in this species may also indicate that it is a marginal host for acacia-ants. Beltian bodies of this species are generally less than 0.6 mm long, commonly are not well developed, and usually occur only on the lower 3–9 pairs of leaflets on some of the pinnae.

According to Janzen (1974), *Acacia chiapensis* occasionally hybridizes with *A. cornigera* and with non-ant-acacias such as *A. macracantha*. During the present study, no specimens were found that would indicate hybridization involving these species. Hybrids involving *A. pennatula* and *A. chiapensis*, however, have been observed.

3. Acacia collinsii Safford, Science N. S. 31: 677. 1910. *Myrmecodendron collinsii* (Saff.) Britton & Rose, N. Amer. Fl. 23: 92. 1928. TYPE: Mexico. Chiapas: between Chicoasén and San Fernandino, 1005 ft., 13 Jan. 1907, *G. N. Collins & C. B. Doyle 180* (holotype, US; photo, F). [Safford (1910) listed Guy N. Collins as the only collector, and cited 14 Jan. 1907 as the date collected; on the label of the herbarium specimen G. N. Collins and C. B. Doyle are listed as the collectors, and 13 Jan. 1907 is given as the date of collection.]

Acacia costaricensis Schenck, Repert. Spec. Nov. Regni Veg. 12: 361. 1913. *Myrmecodendron costaricensis* (Schenck) Britton & Rose, N. Amer. Fl. 23: 93. 1928. TYPE: Costa Rica. Alajuela: Alajuela, 900 m, 1896, *J. D. Smith 6488* (lectotype, designated here, US [B destroyed]; isotypes, BM, GH, K, MO).

Acacia yucatanensis Schenk, Repert. Spec. Nov. Regni Veg. 12: 361. 1913. TYPE: Mexico. Yucatan: 1895, *G. F. Gaumer 353* (lectotype, designated here, US [B destroyed]; isotypes, BM, GH, F, MO).

Acacia panamensis Schenck, Repert. Spec. Nov. Regni Veg. 12: 362. 1913. TYPE: Panama. Aljahuela: Prof. Alex. Koch (holotype, B destroyed).

Acacia nelsonii Saff., J. Wash. Acad. Sci. 4: 363. 1914. TYPE: Mexico. Guerrero: Acapulco, sea level, 30 Apr. 1903, *E. W. Nelson 7024* (holotype, US; isotypes, GH, F).

Acacia penonomensis Saff., J. Wash. Acad. Sci. 4: 363. 1914. TYPE: Panama. Coclé: Penonomé, 50–1000 ft., 23 Feb.–22 Mar. 1908, *R. S. Williams 113* (holotype, NY; photo, F; fragment and photo, US; isotype, US).

Tree to 10 m tall; young twigs reddish brown to dark brown, glabrous. Stipular spines shiny, light reddish brown to dark brown, rarely ivory to yellowish, smooth, glabrous, terete in cross section, mostly symmetrical, V- to U-shaped with an angle of 50–180°, 20–50 mm long, 4–13 mm wide at the base, sometimes reflexed near the tip. Leaves 40–195 mm long; pinnae 3–15 pairs per leaf, 30–90 mm long, 7–17 mm between pinna pairs; rachis grooved, glabrous to lightly puberulent, rachis glands usually absent; petiole grooved, glabrous to lightly puberulent, 4–18 mm long. Petiolar glands 3–5 (rarely 2), dome-shaped to broadly volcano-shaped, usually near the base of the petiole, puberulent, striate, apex 0.3–0.8 mm across, base 1–2.5 mm across. Leaflets 11–29 pairs per pinna, glabrous, linear, 6–13 mm long, 1.3–3.1 mm wide, 2–3 veins from the base, lateral veins obvious, apex obtuse, margins usually not ciliate. Inflorescence of densely flowered cylindrical obtuse spikes, 15–35 mm long, 4–6 mm thick, commonly in short, leafy racemes with 1–3 (rarely 5) spikes per node; peduncles glabrous to lightly puberulent, 6–20 mm long, 0.8–1.8 mm thick, nearly the same thickness throughout; involucre located near the base to lower third of the peduncle, glabrous to lightly puberulent, 4-lobed, the lobes unequal. Floral bracts peltate, apex circular and usually puberulent, stalk 0.6–1.1 mm long. Flowers sessile; calyx 5-lobed, glabrous, 1–1.4 mm long; corolla 5-lobed, glabrous, yellowish, 1.1–1.5 mm long, slightly longer than the calyx. Legumes nearly straight, elliptical in cross section, 30–60 mm long, 7–13 mm wide, glabrous, not striate, dark brown to black, dehiscent along both sutures, not stalked, the apex acute with a short beak 1–6 mm long. Flowering January–August.

Distribution. In shrubby vegetation of pastures and on rocky ridges, in habitats ranging from moderately wet to very dry, at lower elevations (below 1000 m) along the west coast of Mexico from Guerrero east to the Yucatán Peninsula, south

through the dry lowland of Central America to the lowlands of northern Colombia.

Representative specimens. BELIZE. Maskall, *Gentle 1112* (GH, WIS). COLOMBIA. **Bolívar:** Municipio de Arenal, *Forero G. & Jaramillo M. 496* (F). COSTA RICA. **Alajuela:** El Coyolar, 240 m, *Standley 40026* (US). **Guanacaste:** vicinity of Cañas, Finca La Pacífica, *Daubenmire 449* (F). **Puntarenas:** ca. 10 mi. NE of Palmar Sur on the Inter-American hwy., *Janzen 1852* (US). **San José:** Villa Colón, *Janzen 1862* (F, US). EL SALVADOR. **La Unión:** 7.1 mi. W of El Amatillo, *Janzen 1801* (F). GUATEMALA. **Zacapa:** Jones Bridge, 5.8 mi. NE of Río Hondo on hwy. 9, *Janzen 1797* (MEX). HONDURAS. **Colón:** 4.5 mi. NE of Trujillo on old road to Castilla, *Saunders 1040* (F, MO). **Comayagua:** on slope down to Río Selguapa, Comayagua valley, ca. 600 m, *Burch 6033* (MEX, MO). **Olancho:** vicinity of Juticalpa, 380–480 m, *Standley 17522* (F). **Valle:** 28.4 mi. SE of El Amatilla on Inter-American hwy., *Janzen 1841* (F, MEX, US). MEXICO. **Campeche:** 0.8 mi. E of Campeche on hwy. 180, *Janzen 428* (MEX, F). **Chiapas:** along hwy. 190, 13 mi. S of La Trinitaria, 3000 ft., *Breedlove & Raven 8448* (F). **Guerrero:** Petatlán, Montes de Oca, *Hinton 10333* (MO). **Oaxaca:** 11.4 mi. W of Tehuantepec, *Janzen 1780* (F). **Quintana Roo:** 32 mi. N of jct. 307 & 186, N of Chetumal on hwy. 307, *Seigler et al. 11595* (MEX, ILL). **Yucatán:** Chichén Itzá, *Seigler et al. 11599* (EIU, ILL). NICARAGUA. **Esteli:** 1 mi. N Condega, *Janzen 1810* (F). **Rivas:** Peña Blanco, *Janzen 1830* (F, MEX). **Zelaya:** in vicinity of La Luz-Siuna, 150–200 m, *Bunting & Licht 666* (US). PANAMA. **Canal Zone:** Victoria fill, near Miraflores Locks, *Allen 1743* (US). **Coclé:** roadside pasture 20 mi. S of Nata, *Croat 9643* (MO).

Acacia collinsii has the most extensive geographical distribution of all New World ant-acacias and is the only ant-acacia that occurs in South America. It also has the widest ecological distribution, growing in dry to moderately wet pastures and fields and in open shrubby vegetation from sea level to 1000 m. It is a common component of early successional areas. This wide ecological and geographical distribution has resulted in a relatively broad morphological diversity, which is reflected in the extensive synonymy. Though morphologically diverse, it can easily be distinguished from all other ant-acacias by the following combination of characters: elongated cylindrical inflorescences, 3–5 petiolar glands that are broadly dome-shaped, absence of rachis glands, leaflets with obvious lateral veins, and relatively small stipular spines that are terete in cross section.

As is typical of most xerophytic ant-acacias, Beltian body production in *Acacia collinsii* is relatively extensive. In this species the nearly globose Beltian bodies are 0.4–0.8 mm long and usually are found on more than 50% of the leaflets of developing leaves. As most individuals of this species are inhabited by obligate acacia-ants, the Beltian bodies are generally "harvested" soon after development.

Cyanide tests of more than 400 specimens of *Acacia collinsii* indicate that this species is not cyanogenic. Herbarium material of this species was reported to be cyanogenic (Seigler et al., 1978), but reinvestigation of these same specimens failed to confirm activity (Seigler & Ebinger, 1987). Janzen (1981) did not report cyanogenesis for this species.

Acacia collinsii probably hybridizes with *A. hindsii*. These species are sympatric in parts of their ranges in Mexico and Central America, and occasionally occur at the same site. *Acacia collinsii* also hybridizes with the non-ant-acacia *A. pennatula* (Ebinger & Seigler, 1992).

4. **Acacia cookii** Safford, Science N. S. 31: 677. 1910. *Myrmecodendron cookii* (Saff.) Britton & Rose, N. Amer. Fl. 23: 93. 1928. TYPE: Guatemala. Alta Verapaz: Finca Trece Aguas, near Secanguím, 300 m, 8 Mar. 1907, *G. P. Goll 102* (holotype, US).

Acacia bucerophora B. L. Rob., Proc. Amer. Acad. Arts 49: 502. 1913. TYPE: Belize. Belize: about Toledo, 4 mi. from the coast and 5 mi. from Punta Gorda, sea level, 29 Mar. 1907, *M. E. Peck 632* (holotype, GH; fragment and photo, F, US; isotype, F, K).

Tree to 12 m tall, young twigs dark reddish brown to black, densely puberulent. Stipular spines dark brown to black, smooth, terete, densely puberulent, asymmetrical, U-shaped with an angle of 25–50°, usually curved around the stem, 60–100 mm long, 3.5–5.5 mm thick near the base. Leaves 275–500 mm long, pinnae 18–40 pairs per leaf, 10–15 mm between pinna pairs; rachis grooved, densely puberulent; a depressed, elongated gland 1–3 mm long present between each pinna pair; petiole grooved, densely puberulent, 9–16 mm long. Petiolar glands elongate, located in the groove of the petiole, lower than the top of the groove and nearly the same length as the petiole, not striate, glabrous to lightly puberulent, apex 6–13 mm long. Leaflets 50–70 pairs per pinna, glabrous, linear, 6–8 mm long, 0.9–1.2 mm wide, lateral veins not obvious, only one vein from the base, apex acute. Inflorescences of densely flowered globose heads 6–8 mm across, in axillary clusters of 20–70 subtended by normal to slightly reduced leaves; peduncles glabrous to lightly puberulent, 15–30 mm long, 0.8–1 mm thick, slightly thicker near the apex; involucre located near the middle of the peduncle, 4- to 7-lobed, the lobes unequal in length. Floral bracts peltate, apex circular, stalk 0.8–1.1

mm long. Flowers sessile; calyx 5-lobed, glabrous, 1–1.3 mm long; corolla 5-lobed, glabrous, pale yellow, 1.5–1.8 mm long, about ⅓ longer than the calyx. Legumes slightly curved, flattened, 250–300 mm long, 10–16 mm wide, glabrous to lightly puberulent, not striate, dark brown to black, dehiscent along both sutures, stipe 10–40 mm long, the apex narrowing to a terminal point 10–30 mm long. Flowering November–April.

Distribution. In naturally disturbed sites along rivers and in wet forests with a very mild dry season, sea level to 400 m elevation, in Belize and Guatemala.

Representative specimens. BELIZE. Big Rock, Toledo, 200 ft., *Schipp 1072* (F, GH, MICH, MO, NY, UC). GUATEMALA. **Alta Verapaz:** near the Finca Sepacuite, road from Secanguím to Sepacuite, *Cook & Griggs 374* (US). **Izabal:** 2.8 mi. W of Puerto Matías de Gálvez on road to Escobas, *Janzen 1524* (F, GH, MEX, MICH, MO, NY, UC).

Acacia cookii is separated easily from other ant-acacias by its usually asymmetrical, thin, densely puberulent stipular spines that are curved around the stem. The one other species with similar spines, *A. janzenii*, is probably its nearest relative. Though similar, plants of these two species are easily separated. The stipular spines of *A. cookii* are relatively long (60–100 mm), fairly thin (3.5–5.5 mm), and densely puberulent with short gray hairs. *Acacia janzenii*, in contrast, has shorter (30–60 mm), relatively broad (6–9 mm) stipular spines that are densely pubescent with straight, yellowish hairs to 0.5 mm long. In *A. cookii*, the puberulent petioles are 9–16 mm long with a gland to 13 mm long, while in *A. janzenii* the pubescent petioles are less than 8 mm long and lack a gland. The rachis glands that are located between each pair of pinnae also differ in these two species, being elongate (1–3 mm) in *A. cookii* and much smaller (0.5–0.7 mm) and circular in *A. janzenii*. Further, the leaflets of *A. cookii* are 6–8 mm long, usually more than 1 mm wide, and have glabrous margins, while in *A. janzenii* they are shorter (3–5.5 mm), narrower (0.6–0.9 mm), and have ciliate margins. The arrangements of the inflorescences also differ. In *A. cookii* the inflorescences are in axillary clusters of 20–70 on typical branches and are subtended by normal to slightly reduced leaves. In *A. janzenii* some of the inflorescences are on thin fertile branchlets to 250 mm long, with 16–42 inflorescences in each of 6–12 fascicles that are usually not subtended by leaves (Ebinger & Seigler, 1987).

Originally *Acacia cookii* was probably a wet forest species and was commonly found in naturally disturbed sites along rivers in very wet forests (Janzen, 1974). Presently, it is also found along roadsides, in areas of recent landslides, and occasionally in pastures and other sites after human disturbance. According to Janzen (1974), however, this species generally disappears after a forest is cut. Like those of most wet forest ant-acacias, specimens of *A. cookii* generally have relatively low Beltian body production. Commonly only the lower 6–12 pairs of leaflets on a pinna produce Beltian bodies, but usually the bodies are 1–2 mm long. As in most ant-acacias, none of the specimens of this species tested positive for cyanide production.

5. Acacia cornigera (L.) Willdenow, Sp. Pl. 4: 1080. 1806. *Mimosa cornigera* L. Sp. Pl. 520. 1753. *Tauroceras cornigerum* (L.) Britton & Rose, N. Amer. Fl. 23: 86. 1928. TYPE: in the Linnean Herbarium, from a cultivated plant grown in the garden of George Clifford, between Haarlem and Leyden, Holland, collected by Linnaeus (No. 4) and bearing his label "*Mimosa cornigera*," presumably grown from Mexican seed (Rudd, 1964) (holotype, BM; fragment and photo, US).

Acacia cornigera (L.) Willd. var. *americana* DC. Prodr. 2: 460. 1825.

Acacia spadicigera Schltdl. & Cham., Linnaea 5: 594. 1830. *Tauroceras spadicigerum* (Schltdl. & Cham.) Britton & Rose, N. Amer. Fl. 23: 85. 1928. TYPE: Mexico. Veracruz: near La Laguna Verde, Mar. 1828, *Schiede & Deppe 685* (lectotype, designated here, US, fragment [B destroyed]).

Acacia campecheana Schenck, Repert. Spec. Nov. Regni Veg. 12: 361. 1913. TYPE: Mexico. Campeche: von Chrismar (holotype, B destroyed).

Acacia cubensis Schenck, Repert. Spec. Nov. Regni Veg. 12: 360. 1913. TYPE: Cuba. N coast, 21 Apr. 1863, *C. Wright 2402* (lectotype, designated here, US fragment and photo [B destroyed]; isotypes, G, GOET, HAL, JE, K, MO, US). A note on the herbarium sheet (JE) indicates that the seeds he grew came from Martinique.

Acacia interjecta Schenck, Repert. Spec. Nov. Regni Veg. 12: 361. 1913. TYPE: *Engler 3870a* (lectotype, designated here, JE [B destroyed]). Material growing in the Singapore and Kew Botanical Gardens (Janzen, 1974).

Acacia nicoyensis Schenck, Repert. Spec. Nov. Regni Veg. 12: 360. 1913. TYPE: Costa Rica. Guanacaste: shore of the Gulf of Nicoya, sea level, Feb. 1900, *A. Tonduz 13538* (lectotype, designated here, US [B destroyed]; isotypes, BM, GH, K, NY, US).

Acacia rossiana Schenck, Repert. Spec. Nov. Regni Veg. 12: 361. 1913. TYPE: Mexico. Veracruz: Santa Lucrezia, Isthmus of Tehuantepec, 8 Oct. 1906, *H. Ross 918* (lectotype, designated here, M, photo, US).

Acacia furcella Saff., J. Wash. Acad. Sci. 4: 359. 1914. TYPE: Mexico. Veracruz: shore of Lake Catemaco,

southern Veracruz, 1000 ft., 26 Apr. 1894, *E. W. Nelson 427* (holotype, US).

Acacia hernandezii Saff., J. Wash. Acad. Sci. 4: 358. 1914. TYPE: Mexico. San Luis Potosí: vicinity of Rascón, 19–22 July 1905, *E. Palmer 699* (holotype, US; isotypes, F, GH, MO, NY).

Acacia turgida Saff. in W. M. Wheeler, Bull. Mus. Comp. Zoology Harvard Coll. 90: plate 45, 1942 (holotype, plate 45 in Wheeler, 1942).

Shrub or small tree to 10 (rarely 15) m tall, young twigs dark gray to reddish brown, lightly to densely puberulent. Stipular spines light to dark brown to sometimes ivory to yellow, glabrous to densely puberulent, smooth, terete to slightly flattened, symmetrical, commonly V-shaped with an angle of 60–150°, straight to slightly reflexed near the apex, 30–100 mm long, 4–10 mm thick near the base. Leaves 40–160 mm long; pinnae 3–14 pairs per leaf, 30–70 mm long, 7–17 mm between pinna pairs; rachis grooved, glabrous to densely puberulent, rachis glands usually absent; petiole grooved, usually puberulent, 5–20 mm long. Petiolar gland (Fig. 1F) canoe-shaped, usually solitary, glabrous, striate on the sides, apex 1–4 mm long, located near the middle to top of the petiole, sometimes a small tubular gland below. Leaflets 15–40 pairs per pinna, glabrous, oblong, 4–11 mm long, 1.3–2.7 mm wide, 2–3 veins from the base, lateral veins obvious, apex usually mucronate. Inflorescence a densely flowered, cylindrical spike, 20–35 mm long, 8–11 mm thick near the base and narrowing slightly toward the blunt apex, solitary in the leaf axil, or solitary or in clusters of 2–4 in the axil of small spines on short, lateral, usually leafless, axillary branches; peduncles glabrous to lightly puberulent, 5–15 mm long, 2–4 mm thick, thickest just below the inflorescence; involucre usually puberulent, 4-lobed, the lobes spreading, located near the base of the peduncle. Floral bracts peltate, the apex tailed on one side, the stalk 0.7–1.3 mm long. Flowers sessile; calyx shallowly 5-lobed, glabrous to lightly puberulent on the lobes, 1–1.4 mm long; corolla glabrous, pale yellow, 1.1–1.5 mm long, only slightly longer than the calyx. Legume usually straight, mostly terete, 50–90 mm long, 13–18 mm thick, glabrous to minutely puberulent, usually not strongly longitudinally striate, mostly red to maroon, indehiscent, stipe to 10 mm long, the apex narrowing to a spinelike beak 20–50 mm long. Flowering January–July.

Distribution. Wet to relatively dry, mostly disturbed habitats at lower elevations from southern Mexico to Costa Rica.

Representative specimens. BELIZE. Mile 42.5 on Northern Hwy., N of Maskall River, *Dwyer 11023* (F). COSTA RICA. **Guanacaste:** NW of Paloverde, Barbudal Hills, *Garwood et al. 570* (F). EL SALVADOR. Banks of Río Acelhuate, SE part of San Salvador, 690 m, *Carlson 55* (F). GUATEMALA. **Alta Verapaz:** near Pancajche, about 360 m, *Standley 70768* (F). **El Petén:** 2 mi. E of Melchor, roadside, *Croat 24623* (MO). **Escuintla:** near San José at sea level, *Standley 64241* (F). **Retalhuleu:** 9 mi. N of Champerico, *Harmon 2298* (MO). **San Marcos:** 2 mi. E of the border between Mexico (Puente Talisman) on hwy. 2, *Janzen 1045* (F, MEX, MO). **Suchitepéquez:** S of Alotenango Farm, 7 mi. S of Tiquisate along rd. within 3 mi. of ocean, 30–50 m, *Steyermark 47739* (F). HONDURAS. Vegas del Río Agua, 3 km de Yoro, 1000 m, *Molina R. 6807* (F). MEXICO. **Campeche:** 30 km E of Campeche on hwy. 261, *Seigler et al. 11603* (ILL, MEX). **Chiapas:** Ciudad Cuauhtemoc on hwy. 190, *Janzen 499* (F, MEX). **Guerrero:** 1 mi. NW Cuajinicuilapa, *Johnson 740-79* (WIS). **Oaxaca:** Capilla, N end of lake behind Presa Alemán, W of Tierra Blanca, *Janzen 1937* (F, MO, WIS). **Quintana Roo:** 52 mi. W of jct. of Mexico 307 & 186 on hwy. 186, *Seigler et al. 11594* (ILL, MEX). **San Luis Potosí:** Barrio de San Juan, Tamazunchale, *Edwards 600* (F, MO). **Tamaulipas:** Tampico, Rujal rd., *Kenoyer 791* (F). **Veracruz:** Zacuapán, *Purpas 7748* (GH, MO). **Yucatán:** Izarnal, *Greenman 379* (GH). NICARAGUA. Laguna de Masaya, a 2 km de la entrada, *Araquistain & Moreno 593* (MEX).

Acacia cornigera is probably the best known of the ant-acacias. It is easily separated from other ant-acacias by having peltate floral bracts in which the apex is tailed on one side. Also, the presence of canoe-shaped petiolar glands separates this taxon from all ant-acacias except *A. mayana* and *A. sphaerocephala*. The presence of obvious secondary venation in the leaflets and the relatively thick cylindrical inflorescences separate this taxon from *A. sphaerocephala*, while the smaller leaflets and the lack of longitudinal flanges on the stipular spines separate it from *A. mayana*.

Acacia cornigera is a highly variable species that occurs in a wide range of habitats. This morphological diversity has resulted in an extensive synonymy, which is discussed by Rudd (1964). It is the most common of the ant-acacias, and its geographic range is almost as extensive as that of *A. collinsii*. It is relatively common in riparian and swamp habitats and is the common ant-acacia in fallow fields, pastures, roadsides, and other disturbed sites from sea level to about 1200 m (Janzen, 1967a, b). Some of its present distribution has been caused by the dissemination of seeds by birds, people, and cattle into secondary growth vegetation. The present distribution of this species into the drier parts of the Yucatán peninsula is probably due to introduction by humans, since most collections are from around settlements, cattle corrals, and Indian ruins. It has also become naturalized on the Caribbean islands of Martinique, Guade-

loupe, and Cuba, as well as in extreme southern Florida.

Beltian body production in *Acacia cornigera* is typical of that found in ant-acacias that inhabit more open sites. Generally, these bodies are relatively small, 0.5–0.9 mm long, 0.4–0.6 mm wide, and are present on more than half of the leaflets. Since individuals of this species are usually occupied by obligate acacia-ants, the Beltian bodies are rarely seen because they are usually "harvested" as soon as the young leaves develop.

Of the more than 250 herbarium specimens of this species examined, none tested positive for cyanide production. Also, numerous living specimens have been tested, usually with negative results. Leaves of *Acacia cornigera* have been reported to contain a β-glucosidase (Rehr et al., 1973). It appears that the hydrolytic enzyme necessary for the liberation of HCN is present, but the cyanogenic glycoside is absent. Living material from two populations of this species collected near Cañas, Guanacaste Province, Costa Rica, gave a very weak positive test for cyanide (Seigler & Ebinger, 1987). Dried material from these same individuals gave a negative test with and without emulsin.

Janzen (1974) reported seeing a single plant of *A. cornigera* × *A. sphaerocephala* on the dunes south of Veracruz, Mexico, and suggested that *A. cornigera* may occasionally hybridize with *A. chiapensis*. It is also possible that this species may occasionally hybridize with the non-ant-acacia *A. pennatula* (Ebinger & Seigler, 1992).

6. Acacia gentlei Stand., Publ. Field Mus. Nat. Hist., Bot. Ser. 22: 77. 1940. TYPE: Belize. *P. Gentle 185* (holotype, F).

Small tree to 20 m tall; young twigs dark reddish brown, densely puberulent. Stipular spines dull, red to dark brown, most commonly black, usually lightly puberulent, terete, commonly symmetrical to slightly asymmetrical, U-shaped with an angle of 30 to 60°, suberect to recurved around the stem, 35–80 mm long, 3.5–6 mm thick near the base. Leaves 70–180 mm long; pinnae 5–12 pairs per leaf, 55–90 mm long, 13–20 mm between pinna pairs; rachis grooved, puberulent, a narrow, volcano-shaped gland located on the rachis internodes between each pinna pair; petiole grooved, densely puberulent, 9–20 mm long. Petiolar glands narrowly volcano-shaped, usually laterally compressed, solitary (rarely 2), puberulent, lightly striate, apex 0.6–1.2 mm across, base 2–3 mm long, located near the base to middle of the petiole. Leaflets 20–35 pairs per pinna, glabrous, linear, 7–14 mm long, 1.8–3.1 mm wide, lateral veins obvious, 3–5 veins from the base, apex obtuse to acute, margins ciliate. Inflorescence a densely flowered, cylindrical spike 15–40 mm long, 4–6 mm thick, nearly the same thickness throughout, apex blunt, in racemes on fertile branchlets with usually one (occasionally 2–4) spike at a node in the axil of a reduced leaf; peduncles puberulent, 16–22 mm long, 0.7–1.1 mm thick, nearly the same thickness throughout; involucre located near the middle of the peduncle, puberulent, 4-lobed with 2 lobes longer. Floral bracts peltate, apex circular, puberulent, the stalk less than 1 mm long. Flowers sessile; calyx 5-lobed, puberulent, 0.5–0.8 mm long; corolla 5-lobed, puberulent, yellowish, 1.3–1.8 mm long, twice as long as the calyx. Legumes straight to slightly curved, flattened, 160–300 mm long, 9–14 mm wide, glabrous, longitudinally striate, black to dark brown, dehiscent along both sutures, stipe 10–30 mm long, the apex narrowing to a spikelike beak 10–40 mm long. Flowering January–May.

Distribution. Rainforests, wet successional areas where the vegetation is 15–20 years of age, wet disturbed sites, swamps, and river edge vegetation in the lowlands of Belize, northern Guatemala, and extreme southern Mexico.

Representative specimens. BELIZE. 3–6 mi. S of Belmopan, *Dwyer 12544* (F, GH, MO); Southern Hwy., 14 mi. N of Punta Gorda, San Antonio, *Gentry 8072* (MEX, MO); Maskall, *Gentle 1121* (F, GH, MICH, MO, NY); Hummingbird Hwy., *Gentle 9078* (F, MICH, MO, NY); Mountain Pine Ridge, Blancaneaux Lodge, *Wiley 457* (MO). GUATEMALA. **Alta Verapaz:** along Río Sebol between Sebol and Carrizal, N of Sebol, 200–300 m, *Steyermark 45762* (F). **Izabal:** El Zapotillo, 1 km E El Estor (Lake Izabal), *Janzen 657* (F, GH, MEX, MICH, MO, US). **El Petén:** Uaxactun, *Bartlett 12578* (NY, TEX, UC). Tikal National Park, Tikal, in ramonal, *Ibarra 16* (LL). MEXICO. **Chiapas:** a 3 km al S de Frontera Corozal, sobre el Río Usumacinta, 120 m, *Martínez S. 11433* (MEX, MO, NY). **Quintana Roo:** a 2 km al N de Estero Franco, carretera Chetumal–La Unión, *Cabrera & Cabrera 2512* (MEX, MO). **Tabasco:** Carretera W–O por El Poblado de Apatzingan, Balancán, 150 m, *Calzada 2345* (F, MEX, MO).

Acacia gentlei is a common species in wet sites at lower elevations in Belize, Guatemala, and extreme southeastern Mexico. It is distinguished easily from all other ant-acacias by the combination of its elongate inflorescence, laterally compressed, volcano-shaped glands scattered along the leaf rachis, very thin spines (usually less than 6 mm thick), and relatively large leaflets (7–14 mm long) with obvious secondary veins.

Unlike many of the wet forest ant-acacias, *Acacia gentlei* is a common species in areas of human

disturbance. It appears as a successional species after logging, and in many parts of its range is found along roadsides. Unlike most wet forest ant-acacias, it has relatively large Beltian bodies (1–2 mm long) on most of the leaflets. Like most of the other ant-acacias, it usually lacks cyanogenic glycosides in the leaflets. Of the specimens examined, only one, *Lundell 16903*, from Guatemala, was weakly cyanogenic.

7. **Acacia globulifera** Saff., J. Wash. Acad. Sci. 4: 360. 1914. *Myrmecodendron globuliferum* (Saff.) Britton & Rose, N. Amer. Fl. 23: 93. 1928. TYPE: Mexico. Yucatán: at the port of Silam (Tzilam), N coast of Yucatán, Apr. 1895, *G. F. Gaumer 1909* (holotype, F, fragment and photo, US; isotypes, GH, MO, NY, US).

Acacia donnelliana Saff., J. Wash. Acad. Sci. 4: 361. 1914. *Myrmecodendron donnellianum* (Saff.) Britton & Rose, N. Amer. Fl. 23: 93. 1928. TYPE: Honduras. Santa Bárbara: San Pedro de Sula, Cortes, 600 ft., Mar. 1888, *C. Thieme 5216* (holotype, US).

Shrub or small tree usually less than 3 (rarely 10) m tall, young twigs brown to reddish brown, glabrous to lightly puberulent. Stipular spines (Fig. 1B) usually dark brown to black (sometimes yellow to ivory), smooth, terete, glabrous to lightly puberulent, symmetrical, V-shaped with an angle of 40–120°, straight to reflexed near the apex, 25–60 mm long, 4–7 mm thick near the base. Leaves 60–250 mm long; pinnae 6–26 pairs per leaf, 30–65 mm long, 6–11 mm between pinna pairs, rachis grooved, glabrous to lightly puberulent, a small volcano-shaped gland present between each pinna pair; petiole grooved, glabrous to lightly puberulent, 8–18 mm long. Petiolar glands (Fig. 1C) volcano-shaped, commonly laterally compressed, densely puberulent, usually lightly striate, apex nearly circular, 0.3–0.9 mm across, base 1–2 mm across, usually 2–5 glands scattered along the petiole. Leaflets 20–50 pairs per pinna, glabrous, linear, 4–6 mm long, 0.6–1.2 mm wide, lateral veins not obvious, only one vein from the base, apex mostly acute. Inflorescence a densely flowered globose head 4–7 mm across, in clusters of 4–8 in the axil of slightly reduced leaves, or in small clusters in the axil of small spines on lateral, usually leafless branches; peduncles glabrous to lightly puberulent, 6–25 mm long, 0.5–1.0 mm thick, nearly the same thickness throughout; involucre located on the lower ¼ of the peduncle, glabrous to lightly puberulent, 4-lobed. Floral bracts peltate, apex circular, the stalk about 1 mm long. Flowers sessile; calyx 5-lobed, glabrous, 1–1.5 mm long; corolla 5-lobed, glabrous, pale yellow, 1.5–2.0 mm long. Legumes slightly curved, elliptical to nearly terete in cross section, 50–90 mm long, 9–12 mm wide, glabrous, not striate, black to dark brown, dehiscent along one suture, stipe less than 5 mm long, the apex narrowing to a terminal spinelike beak usually less than 10 mm long. Flowering January–April.

Distribution. In riparian and swamp successional stages as well as open, dry habitats, from sea level to about 1200 m, in southern Mexico (Campeche, Oaxaca, Yucatán), Belize, Guatemala, and Honduras.

Representative specimens. BELIZE. **El Cayo:** Vaca, *Gentle 2275* (AA, F, MICH, MO, NY). GUATEMALA. **Amatitlán:** Laguna (Lake Amatitlán), 1200 m, *Kellerman 5042* (UC). **Guatemala:** 20.5 mi. NE Guatemala City on hwy. 9, *Janzen 1565* (F, GH, MEX, MICH, MO, US). **El Petén:** in Mananche, *Contreras 5480* (F, MICH, US). **Zacapa:** along Río Teculután, above Teculután, 250–275 m, *Steyermark 42146* (F, NY). HONDURAS. Cut over river valley lands, Yoro 2800 ft., *Edwards P-748* (AA, F); 26 mi. SW of San Pedro Sula on hwy. 18, *Janzen 1608* (F); 10.4 mi. SW of Santa Rosa on hwy. 18, *Janzen 1634* (F, MICH, MO, UC, US). MEXICO. **Campeche:** 48 mi. NE of Puerto Real (Isla Aguada) on hwy. 180, *Janzen 611* (F, MICH, US). **Oaxaca:** 11.3 mi. N of intersection of Mexico hwy. 185 and 190, on hwy. 185, *Janzen 1502* (F, GH, MEX, MICH, MO, NY, UC, WIS). **Quintana Roo:** en San José de la Montaña, km 8 de la carreterra a Tomás Garrido, *Cabrera et al. 4504* (MEX). **Yucatán:** Silám, *Gaumer 655* (F, GH, US).

Acacia globulifera is easily distinguished from most ant-acacias by its spherical inflorescences, small leaflets that lack obvious secondary veins, and 2–5 narrow volcano-shaped petiolar glands. It is most closely related to *A. chiapensis* and has been occasionally combined with it (Janzen, 1974). These two species differ, however, in petiolar glands, inflorescence clusters, and fruit characteristics (see discussion under *A. chiapensis*). *Acacia globulifera* is commonly found in riparian or relatively dry sites from sea level to about 1200 m, which is the upper elevation limit of ant-acacias. It usually grows in open, fully insolated habitats, rarely exceeds 3 m in height, and is usually restricted to young successional habitats (Janzen, 1974).

Beltian body production in *Acacia globulifera* is typical of that found in most ant-acacias that inhabit more open sites. These bodies, which are less than 0.8 mm long, usually are present on more than half of the leaflets of a developing leaf. The bodies are rarely seen, as they are usually "harvested" soon after development by obligate acacia-ants.

Acacia globulifera is similar to *A. chiapensis*

in that many individuals of both species are cyanogenic; the cyanogenic glycoside of *A. globulifera* is (*R*)-epiproacacipetalin, whereas that of *A. chiapensis* is (*S*)-proacacipetalin. Of the specimens of *A. globulifera* tested, most gave a positive test for HCN. Of these specimens, however, nearly one-third required the addition of emulsin to give a positive test. This suggests that many individuals of this species either lack the enzyme capable of hydrolyzing the cyanoglycoside, or that the enzyme is inactivated by drying and storage (Seigler & Ebinger, 1987).

Janzen (1974) suggested that *Acacia globulifera* may occasionally hybridize with a non-ant-acacia of the *A. macracantha* complex. During the present study no specimens were found that would indicate hybridization involving this species.

8. Acacia hindsii Benth., London J. Bot. 1: 504. 1842. *Myrmecodendron hindsii* (Benth.) Britton & Rose, N. Amer. Fl. 23: 91. 1928. TYPE: Mexico. Jalisco: shore of Manzanilla Bay, sea level, 1841, *R. B. Hinds 248* (holotype, K, photo, F).

Acacia bursaria Schenck, Repert. Spec. Nov. Regni Veg. 12: 363. 1913. TYPE: Guatemala. Amatitlán: Laguna Amatitlán, 3900 ft., Feb. 1890, *J. D. Smith 2304* (lectotype, designated here, US [B destroyed]; isotypes, GH, K).

Acacia tepicana Saff., J. Wash. Acad. Sci. 4: 366. 1914. TYPE: Mexico. Nayarit: thickets, vicinity of Acaponeta, Tepic, 30 m, 10 Apr. 1910, *J. N. Rose, P. C. Standley & P. G. Russell 14357* (holotype, US, photo, F; isotype, NY).

Acacia sinaloensis Saff., J. Wash. Acad. Sci. 4: 365. 1914. TYPE: Mexico. Sinaloa: vicinity of Villa Unión, growing about a pond, 2 Apr. 1910, *J. N. Rose, P. C. Standley & P. G. Russell 13972* (holotype, US; isotype, NY).

Tree to 10 m tall; young twigs reddish brown to dark brown, glabrous to rarely lightly puberulent. Stipular spines (Fig. 1C) shiny, light brown to nearly black (sometimes light gray), smooth, glabrous to lightly puberulent, flattened at the base, symmetrical, nearly flat to broadly U-shaped across the top at an angle of 90–180°, 30–55 mm long, 10–20 mm wide at the base. Leaves 45–180 mm long; pinnae 10–18 pairs per leaf, 20–45 mm long, 6–12 mm between pinna pairs; rachis grooved, usually puberulent, a small narrow volcano-shaped gland located at the node between each pinna pair; petiole grooved, usually densely puberulent, 7–14 mm long. Petiolar glands narrow volcano-shaped (to almost columnar), usually 3–7 (rarely 1) scattered along the petiole, puberulent, lightly striate, apex 0.4–0.7 mm long, base 0.8–1.2 mm long. Leaflets 12–30 pairs per pinna, glabrous, linear, 3–7 mm long, 0.9–1.4 mm wide, one vein from the base, lateral veins not obvious, apex obtuse, margins lightly ciliate. Inflorescence a loosely flowered, cylindrical spike, 20–50 mm long, 4–7 mm thick, nearly the same thickness throughout, apex blunt, in racemes with 1–3 (rarely 4–8) spikes at each node in the axil of a reduced leaf; peduncles glabrous to lightly puberulent, 10–20 mm long, 0.6–1.1 mm thick, nearly the same thickness throughout; involucre located at the base to the lower third of the peduncle, puberulent, 4-lobed with 2 lobes longer. Floral bracts peltate, apex circular, stalk 0.4–0.7 mm long. Flowers sessile; calyx 5-lobed, puberulent, 0.5–0.9 mm long; corolla 5–6-lobed, puberulent, yellowish, 1.6–2.0 mm long, usually more than twice as long as the calyx. Legumes curved, elliptical in cross section, 40–100 mm long, 8–12 mm wide, glabrous to lightly puberulent, usually not striate, black to dark brown, dehiscent along one suture, short stalked, base narrowly cuneate, the apex narrowing to a beak 10–15 mm long. Flowering January–July.

Distribution. Disturbed, usually wet sites of the Pacific lowlands and foothills from extreme southern Sinaloa, Mexico, south to Nicaragua.

Representative specimens. EL SALVADOR. **La Unión:** vicinity of La Unión, 150 m or less, *Standley 20663* (NY, UC, US). **San Vicente:** vicinity of Apastepeque,, *Standley 21333* (NY). **Sonsonate:** vicinity of Acajutla, 30 m or less, *Standley 21942* (US). GUATEMALA. **Alta Verapaz:** near Pancajche, about 360 m, *Standley 70648* (F). **Escuintla:** S of Río Burrion, NE of Escuintla, about 700 m, *Standley 89619* (F). **Retalhuleu:** vicinity of Retalhuleu, 240 m, *Standley 88846* (F). **San Marcos:** Sands, Ocos, 1–2 m, *Steyermark 37779* (F). HONDURAS. Encinales del Valle de El Espino cerca de San Jerónimo, 350 m, *Molina R. 8059* (F, MO, US). MEXICO. **Chiapas:** 13 mi. NE of the border of Chiapas & Oaxaca on hwy. 190, *Seigler et al. 11583* (ILL). **Colima:** Manzanillo, *Palmer 1395* (MO, NY, US). **Guerrero:** sandy river bank, Tecpan–El Verde, 20 m, *Hinton 14120* (MO, TEX, US). **Jalisco:** just E of Barra de Navidad in sandy soil near sea level, in thickets about margin of palm forest, *McVaugh 11847* (US). **Michoacán:** a 10 km aprox. al N de Playa Azul, carr. a Nueva Italia, *Nuñez & Boom 2115* (MO). **Nayarit:** 4 mi. S of intersection to Acaponeta on hwy. 15, *Seigler et al. 11797* (ILL). **Oaxaca:** sand dunes behind the beach at Salina Cruz, *King 2461* (TEX, US). **Sinaloa:** near Colomas, in the foothills of the Sierra Madre, *Rose 1766* (NY).

Like *Acacia collinsii* and *A. cornigera*, *A. hindsii* has an extensive geographical range. Unlike these species, however, *A. hindsii* is restricted to the Pacific lowlands and foothills in Central America. Before the existence of extensive agri-

culture in the region it was probably common along rivers, in semideciduous and deciduous forests, and in mangrove swamps (Janzen, 1974). Presently it is a common element of shrubby regeneration, particularly in wetter habitats such as river banks, where it commonly forms dense thickets by means of root sprouts.

Acacia hindsii is easily separated from all other ant-acacias by its stipular spines, which are flattened at the base and nearly flat to broadly U-shaped across the top. It is morphologically very similar to *A. collinsii* and *A. gentlei*, both of which have relatively small leaves and cylindrical spikes, but differs in leaflets that lack obvious secondary venation.

As is typical of most ant-acacias that inhabit more open sites, Beltian body production in *Acacia hindsii* is relatively extensive. In this species the small, slightly elongated Beltian bodies usually are less than 1 mm long, and are found commonly on more than 60% of the leaflets of developing leaves. As most individuals of this species are inhabited by obligate acacia-ants, the Beltian bodies generally are not seen, being "harvested" soon after development.

Acacia hindsii is polymorphic with respect to HCN production (Seigler & Ebinger, 1987), being reported acyanogenic by Rehr et al. (1973), while Seigler et al. (1978) found individuals that are strongly cyanogenic. Of more than 300 herbarium specimens tested, 123 were positive for HCN production. In general, many of the specimens from Mexico tested positive for cyanide, while most of the acyanogenic specimens are from Guatemala, El Salvador, and Honduras. The glycoside in this species is proacacipetalin.

Acacia hindsii probably hybridizes with the ant-acacia *A. collinsii*. It also has been reported to hybridize with non-ant-acacias of the *A. macracantha* complex, particularly *A. pennatula* (*Acacia* × *standleyi* Safford), and *A. cochliacantha* (*Acacia* × *gladiata* Safford).

9. Acacia janzenii Ebinger & Seigler, Southw. Naturalist. 32: 245. 1987. TYPE: Mexico. Tabasco: 9.8 mi. W of Lázaro Cárdenas on hwy. 180, 17 June 1966, *D. H. Janzen 515* (holotype, MO; isotypes, BM, CAS, F, GH, MICH, MO, UC, US).

Tree to 12 m tall, young twigs dark reddish brown, lightly puberulent. Stipular spines dark brown to black, smooth, terete, densely pubescent with straight, yellowish hairs to 0.5 mm long, asymmetrical, U-shaped with an angle of 30–70°, usually curved around the stem, 30–60 (rarely 80) mm long, 6–9 mm thick near the base. Leaves 150–370 mm long; pinnae 30–60 pairs per leaf, 6–9 mm between pinna pairs; rachis grooved, densely pubescent with yellowish hairs, a columnar to narrowly volcano-shaped gland located at the node of each pinna pair, its apex 0.5–0.7 mm across; petiole densely pubescent, 5–8 mm long. Petiolar glands absent. Leaflets 40–70 pairs per pinna, glabrous, linear, 3–5.5 mm long, 0.6–0.9 mm wide, lateral veins not obvious, only one vein from the base, apex acute, margins ciliate. Inflorescence a densely flowered globose head 4–6 mm across, in clusters of 16–42 in the axil of slightly reduced leaves on normal branches, also located on fertile branchlets to 250 mm long with 16–42 inflorescences in each of 6–12 fascicles that may not be subtended by leaves; peduncles glabrous to lightly puberulent, 7–16 mm long, 0.3–0.7 mm thick, the same thickness throughout; involucre located near the middle of the peduncle, usually 4-lobed. Floral bracts peltate, apex circular, stalk 0.4–0.7 mm long. Flowers sessile, calyx 5-lobed, glabrous, 0.6–1.1 mm long; corolla 5-lobed, glabrous, 1.2–2.1 mm long, about twice as long as the calyx. Legumes not seen, but probably very similar to those of *Acacia cookii*. Flowering May–July.

Distribution. Disturbed habitats, roadsides, pastures, and moist disturbed forests, to 400 m elevation, in Chiapas, Tabasco, and Veracruz, Mexico.

Representative specimens. MEXICO. **Chiapas:** stream bank, lower montane rainforest ridge above Ixtacomitán, 300 m, *Breedlove 35053* (MEX, MICH, MO). **Tabasco:** 9.8 mi. W of Cárdenas on hwy. 180, *Janzen 515* (MEX). **Veracruz:** Las Cruces, 250 m, *Nevling & Gómez-Pompa 1555* (F).

Acacia janzenii is easily distinguished from all other ant-acacias by its asymmetrical stipular spines, which usually curve around the stem and are covered with straight, yellowish hairs to 0.5 mm long. The only other species that commonly has asymmetrical thorns is *A. cookii*. These two closely related species differ in the petiolar and rachis glands, the type of pubescence on the spines, the leaflet length and width, and the flowering branchlets (see discussion under *A. cookii*).

Janzen (1974) considered this taxon a part of *Acacia cookii*. However, he noted that specimens from Tabasco and Chiapas, Mexico (herein referred to *A. janzenii*) differ from typical *A. cookii*. In particular, he noted that progressing from north (Mexico) to south (Belize, Guatemala, Honduras),

there is a change in spine length, a shortening of the fertile branchlets, an increase in the number of inflorescences per axil, an increase in the number of Beltian bodies per leaf, and a higher percentage of plants occupied by ants. Although he suggested that the extremes are clinally connected, our principal component analysis (PCA) suggests that these two species are specifically distinct. No intermediate plants were found, and the characters used for separation (petiolar and rachis glands, spine size, pubescence, leaflet size, fertile branchlets) are as significant as those characters used to distinguish other taxa of ant-acacias.

Originally, *Acacia janzenii* probably was a species of disturbed sites in open forests, particularly landslide scars and stream banks. Most recent collections seen are from roadsides and pastures.

Beltian body production in this species is relatively low; usually less than ¼ of the leaflets contain these bodies. Also, these bodies are usually less than 0.7 mm long. None of the specimens tested positive for cyanide production.

10. Acacia mayana Lundell, Publ. Carnegie Inst. Wash. 478: 210. 1937. TYPE: Guatemala. El Petén: near San Diego on the Río Pasión, 10 Apr. 1935, *M. Aguilar H. 495* (holotype, MICH; isotypes, GH, NY, US).

Shrub or small tree to 10 m tall; young twigs gray to light brown, glabrous. Stipular spines shiny, dark black, glabrous, symmetrical, V-shaped with an angle of 70 to 150°, the upper half strongly reflexed, 30–75 mm long, 5–12 mm thick near the base, two bladelike longitudinal flanges extending from the base to the apex along each side of the spine. Leaves 150–400 mm long; pinnae 6–19 pairs per leaf, 75–120 mm long, 15–30 mm between pinna pairs; rachis grooved, glabrous to puberulent, a small, elongated gland present between each pinna pair; petiole grooved, glabrous to puberulent, 15–35 mm long. Petiolar glands canoe-shaped, solitary (rarely 2), glabrous, striate on the sides, apex 1.2–5 mm long, located just below the first pinna pair. Leaflets 25–40 pairs per pinna, glabrous, linear, 10–23 mm long, 1.8–3.5 mm wide, lateral veins obvious, 3–5 veins from the base, apex obtuse. Inflorescence a densely flowered spike, 30–50 mm long, 7–10 mm near the base, narrowing toward the elongated and pointed apex, solitary or in small racemes on short, leafless, axillary branches; peduncles glabrous, 5–15 mm long, 4–6 mm thick, nearly the same thickness throughout; involucre located near the base of the peduncle, glabrous to lightly puberulent, with 4–5 irregular, shallow lobes. Floral bracts peltate, apex circular, the stalk 0.9–1.2 mm long. Flowers sessile; calyx shallowly 5-lobed, glabrous, 0.9–1.2 mm long; corolla 5-lobed, glabrous, pinkish, 1–1.3 mm long, only slightly longer than the calyx. Legume slightly curved, nearly terete, 90–120 mm long, 12–15 mm thick, glabrous, longitudinally striate, dark reddish brown, indehiscent, stipe to 25 mm long, the apex narrowing to a long spinelike beak 7–20 mm long. Flowering January–June.

Distribution. Apparently a species of lowland, wet forests and forest margins in the departments of Alta Verapaz and El Petén, Guatemala, and the states of Chiapas, Oaxaca, Tabasco, and Veracruz, Mexico.

Representative specimens. GUATEMALA. **Alta Verapaz:** along Río Icuolay, N and NW of Finca Cubilguitz to Quebrada Diablo, 300–350 m, *Steyermark 44726* (F). **El Petén:** high forest in zapotal & corozal, Chinchila, Sebol road, *Contreras 10691* (F). MEXICO. **Chiapas:** near Lacanjá, Mpio. Ocosingo, *Breedlove 34505* (MEX). **Oaxaca:** San Felipe, a 7 km al N–NW de Macedonio Alcalá, Distr. de Tuxtepec, 80 m, *Sousa et al. 7286* (MEX, MICH, NY). **Tabasco:** Retiro, above Tenosique, in virgin forest, *Matuda 3410* (F, GH, LL, MEX, MICH, MO, NY). **Veracruz:** Estación de Biología Tropical Los Tuxtlas, 200 m, *Ibarra M. 1449* (MO, NY).

According to Janzen (1974), *Acacia mayana* probably represents a "wet-forest edition" of *A. cornigera*. Undoubtedly, the two taxa are very closely related, having many vegetative and floral characteristics in common. However, the large leaflets (more than 10 mm long), the rachis glands between each pinna pair, and the inflorescence, which narrows toward the elongated and pointed apex, separate this species from the closely related *A. cornigera* and *A. sphaerocephala*. Also, the pair of bladelike longitudinal flanges extending from the spine base to apex separates *A. mayana* from all other species of ant-acacia.

Acacia mayana is one of the rarest of the ant-acacias. Collecting data from the few collections observed indicate that it has pinkish flowers and varies in size from a shrub to a small tree to 10 m tall. Most collections indicate that it occurs as widely scattered individuals in moist lowland forests. Janzen (1974) reported an individual from an old second growth cornfield regeneration where the forest was about 15 m tall.

Unlike most wet forest ant-acacias, Beltian body production in *Acacia mayana* is extremely high. On developing leaves, nearly all of the leaflets contain Beltian bodies, and these bodies are usually about 2 mm long and up to 0.8 mm wide. As is typical of most ant-acacias, none of the individuals

of *A. mayana* tested positive for cyanide production.

11. Acacia melanoceras Beurl., Kongl. Svenska Vetensk. Acad. Handl. 1854: 123. 1856. *Myrmecodendron melanoceras* (Beurl.) Britton & Rose, N. Amer. Fl. 23: 93. 1928. TYPE: Panama. Colón: Portobello, sea level, Apr. 1826, *J. G. Billberg 289* (lectotype, designated here, S, photo, F).

Acacia multiglandulosa Schenck, Repert. Spec. Nov. Regni Veg. 12: 362. 1913. TYPE: Panama. Colón: Portobello, *J. G. Billberg 1825 (lectotype, designated here, US, photo [B destroyed]).*

Tree to 15 m tall; young twigs dark brown to dark reddish brown, puberulent. Stipular spines shiny, dark reddish brown to black, grooved and with low, rounded longitudinal ridges, rarely with two narrow, bladelike longitudinal flanges, glabrous to lightly puberulent, terete in cross section, symmetrical, broadly V-shaped with an angle of 80–120°, 20–55 mm long, 5–9 mm wide near the base, abruptly tapering to a narrow, sharp-pointed tip, many spines not enlarged, usually less than 5 mm long and 0.4 mm wide. Leaves 150–290 mm long; pinnae 12–28 pairs per leaf, 20–50 mm long, 6–11 mm between pinna pairs; rachis grooved, puberulent; rachis glands puberulent, striate, columnar to narrowly volcano-shaped, one located at the node of each pinna pair, its apex 0.2–0.6 mm across; petiole grooved to flattened on the adaxial surface, densely puberulent, (5)10–28 mm long. Petiolar glands columnar to narrowly volcano-shaped, usually numerous (6–30) on the flattened adaxial surface of the petiole, reduced to 0–5 on petioles of smaller leaves, puberulent, striate, apex 0.3–1.1 mm across, base 0.4–1.5 mm across. Leaflets 12–29 pairs per pinna, glabrous, linear, 4.5–7.6 mm long, 1.0–1.7 mm wide, lateral veins not obvious, only one vein from the base, apex acute. Inflorescence a densely flowered globose head 5–6.5 mm across, solitary or in clusters of 2–6 in the axil of small spines on lateral, usually leafless, axillary branches to 350 mm long; peduncles densely puberulent, 5–10 mm long, 0.5–0.8 mm thick, nearly the same thickness throughout; involucre located near the base of the peduncle, puberulent, 5-lobed. Floral bracts peltate, apex circular, ciliate, stalk 0.8–1.2 mm long. Flowers sessile; calyx 5-lobed, glabrous, 0.9–1.1 mm long; corolla 5-lobed, glabrous, pale yellow, 1.5–1.8 mm long. Legumes curved, particularly at the narrowing apex, inflated to slightly flattened, 65–110 mm long, 10–20 mm wide, glabrous, longitudinally striate, dark brown to black, indehiscent, stipe absent, the apex narrowing and usually acuminate. Flowering April–July.

Distribution. Wet, relatively undisturbed forests on the Atlantic lowlands of central Panama (Colón Province) and the Canal Zone.

Representative specimens. PANAMA. **Colón:** Río Boquerón, about 6–8 km upstream from Peluca Hydrographic Station, *Dressler 4657* (F, GH, MO); 500 m upstream from mouth of Río Piedras, *Janzen 1641, 1912, 1913, 1915, 1916* (F, GH, MEX, MO). **Canal Zone:** Barro Colorado Island, *Aviles 20b* (F, MO); along Río Mendosa near Pipeline Road bridge, 8 km NW of Gamboa, 95 m, *Nee 7739* (MO).

Acacia melanoceras has the most restricted range of all ant-acacias and is relatively rare throughout this range. According to Janzen (1974), rarely more than two individuals are found per acre in forest communities. Also, this species, which is usually restricted to lowland wet forests, disappears from any habitat subjected to disturbances that are any more catastrophic than infrequent cutting.

This species can easily be distinguished from all other ant-acacias by the extremely high number (6–30) of volcano-shaped petiolar glands on the flattened adaxial surface of the petiole. Also, the large leaves (to 300 mm) and the rachis glands at the node of each pinna pair help to separate it from most other ant-acacias.

Beltian body production is relatively low in *Acacia melanoceras*, rarely more than half of the leaflets on mature plants producing these structures, which vary from 1 to 1.6 mm long. This characteristic, which is typical of most wet forest ant-acacias, is probably due to the high cost to the plant of Beltian bodies in a habitat with low solar energy input and perhaps somewhat reduced herbivore loads (Janzen, 1974). None of the individuals of this species tested positive for cyanide production.

12. Acacia ruddiae D. H. Janzen, Smithsonian Contr. Bot. 13: 34. 1974. TYPE: Costa Rica. Limón: ferry landing, Moin River, Moin, 13 Feb. 1965, *D. H. Janzen 828* (holotype, US; isotypes, CAS, F, GH, MEXU, MICH, MO, NY, UC).

Tree to 30 m tall; young twigs dark brown to dark reddish brown, glabrous to puberulent. Stipular spines dull, dark reddish brown to black, smooth, glabrous to lightly puberulent, nearly terete in cross section, symmetrical, broadly V-shaped

with an angle of 70–180°, to 9.5 mm long, 4–9 mm wide, abruptly tapering to a narrow, sharp-pointed tip; many spines not enlarged, usually less than 8 mm long and to 0.4 mm wide. Leaves 130–400 mm long; pinnae 17–40 pairs per leaf, 30–55 mm long, 5–13 mm between pinna pairs; rachis grooved, densely puberulent; rachis glands glabrous, striate, cylindrical, commonly narrowing toward the base, to 1 mm long, one located at the node of each pinna pair, its apex 0.4–0.9 mm across; petiole grooved, densely puberulent, 6–25 mm long. Petiolar glands commonly absent, rarely 1 or 2 present, identical to the rachis glands. Leaflets 25–75 pairs per pinna, glabrous, ciliate along the margins, linear, 3.5–6.5 mm long, 0.6–1.1 mm wide, lateral veins not obvious, only one vein from the base, apex acute. Inflorescence a densely flowered globose head 6–7 mm across, in clusters of 1–8 in the axil of normal leaves; peduncles densely puberulent, 8–11 mm long, 0.5–0.7 mm thick, nearly the same thickness throughout; involucre located near the middle of the peduncle, puberulent, 5-lobed. Floral bracts peltate, apex circular, ciliate, stalk less than 0.8 mm long. Flowers sessile; calyx 5-lobed, glabrous, 0.7–1.0 mm long; corolla 5-lobed, glabrous, pale yellow, 1.3–1.6 mm long. Mature legumes not seen. Flowering July.

Distribution. Very wet rainforest, particularly in primary succession along rivers, at edge of swamps, and on landslide scars in the Caribbean lowlands, from Puerto Barrios area of Guatemala to northern Panama.

Representative specimens. COSTA RICA. **Alajuela:** 11 mi. N Florencia, *Janzen 1883* (F), *1885* (F), *1886* (F, MICH). **Heredia:** Finca La Selva, the OTS Field Station on the Río Puerto Viejo just E of its junction with the Río Sarapiquí, 100 m, *Hammel & Trainer 12918* (MICH, MO); 16 mi. N of Vara Blanca on rd. from Heredia to Puerto Viejo, *Janzen 819* (F, GH, MICH, NY, US). **Limón:** 1 km W of La Lola (on Limón–Siquirres railroad), *Janzen 1868* (F, NY), *1872* (F), *1874* (F), *1875* (F), *1877* (F, NY, US), *1881* (F). GUATEMALA. **Izabal:** 1.9 mi. W of Puerto Matias de Gálvez (on rd. to Escobas), *Janzen 1582* (F, GH, MEX, MICH, US). HONDURAS. **Atlántida:** Lancetilla Valley, near Tela, 20–600 m, *Standley 54627* (F). NICARAGUA. **Zelaya:** Municipio de Rama, *Robleto 664* (MO). PANAMA. **Bocas del Toro:** Campamento forestal, Boca Chica, *Correa A. et al. 3581* (MO, NY); along Changuinola River, Changuinola Valley, *Dunlap 462* (F).

Acacia ruddiae is probably most closely related to *A. cookii* and *A. janzenii*, which it resembles in having small globose inflorescences, large leaves with numerous small leaflets, and rachis glands between each pinna pair. However, both *A. cookii* and *A. janzenii* have asymmetrical stipular spines that usually curve around the stem, whereas *A. ruddiae* has symmetrical spines. Also, the rachis glands of *A. ruddiae* are urn-shaped, narrowing slightly toward the base, whereas in *A. cookii* and *A. janzenii* they are elongated to volcano-shaped.

Though *Acacia ruddiae* has a relatively extensive geographical range in the Caribbean lowlands from Guatemala to Panama, it has rarely been collected. The presence of this species in Costa Rica was discussed by Calvert & Calvert (1917) who mentioned a bull-horn acacia with swollen thorns and urn-shaped rachis glands that lacked Beltian bodies and obligate acacia-ants. Though referring to this species as *Acacia campeachiana* Miller (= *A. cochliacantha*), there is little doubt that the taxon discussed is *A. ruddiae*.

This wet forest species is only marginally an ant-acacia. Janzen (1974) found that relatively few of the stipular spines were swollen, none were occupied by obligate acacia-ants, and Beltian bodies were not formed on the leaflets. During the present study, no indication of Beltian bodies was found on developing leaves; all of the mature leaves examined lacked the scar left by these bodies. However, a characteristic entrance hole made by ants was observed on two specimens (*Correa A. et al. 3581* and *Janzen 819*). These probably represent chance encounters, as none of the other spines on the specimens had entrance holes.

Of the specimens of *Acacia ruddiae* examined, none tested positive for HCN production. Seigler & Ebinger (1987) obtained similar results, finding one individual that was weakly cyanogenic while the rest tested negative.

13. Acacia sphaerocephala Schltdl. & Cham., Linnaea 5: 594. 1830. TYPE: Mexico. Veracruz: Actopan, sea level, Mar. 1829, *Schiede & Deppe 684* (holotype, HAL, fragment and photo, F, US).

Acacia veracruzensis Schenck, Repert. Spec. Nov. Regni Veg. 12: 362. 1913. TYPE: Mexico. Veracruz: sand dunes S of Veracruz, sea level, 13 Oct. 1908, culta in hort. bot. Darmstadt., *H. Schenck 916* (lectotype, designated here, M, fragment and photo, US [B destroyed]; isotypes, F, HAL).

Acacia dolichocephala Saff., J. Wash. Acad. Sci. 5: 355. 1915. TYPE: Mexico. Veracruz: along the shore N of the city of Veracruz, 24 Jan. 1906, *J. M. Greenman 87* (holotype, F; isotypes, GH, NY, US, photo, K).

Small, spreading, much-branched shrub to 2 (rarely 5) m tall; young twigs dark gray to reddish brown, glabrous to lightly puberulent. Stipular spines ivory to yellow or rarely reddish to dark brown,

glabrous to lightly puberulent, smooth, terete, mostly symmetrical, V-shaped, straight to slightly reflexed, 20–80 mm long, 6–16 mm thick near the base. Leaves 40–140 mm long; pinnae 5–15 pairs per leaf, 20–55 mm long, 7–17 mm between pinna pairs; rachis grooved on the upper surface, glabrous to lightly puberulent, rarely a few glands present; petiole grooved, glabrous to lightly puberulent, 6–13 mm long. Petiolar glands canoe-shaped, solitary, glabrous, striate on the sides, apex 1.4–4.5 mm long, located near the middle of the petiole, sometimes a small tubular gland below. Leaflets 16–48 pairs per pinna, glabrous, oblong, 4–10 mm long, 1.4–2.2 mm wide, lateral veins not obvious, apex mucronate. Inflorescence a densely flowered, subglobose spike less than 2× longer than wide, 7–14 mm long, 5–7 mm wide, apex blunt, solitary or in clusters of 2–6 in the axil of small spines on short, usually leafless, axillary branches; peduncles glabrous to lightly puberulent, 7–18 mm long, 0.8–1.1 mm thick, nearly the same thickness throughout; involucre located at the base of the peduncle, glabrous to lightly puberulent, 4-lobed. Floral bracts peltate, apex circular, the stalk 0.9–1.5 mm long. Flowers sessile; calyx 5-lobed, glabrous to lightly puberulent, 1–1.5 mm long; corolla 5-lobed, glabrous, pale yellow, 1.2–1.8 mm long, only slightly longer than the calyx. Legumes straight, nearly terete, 30–80 mm long, 12–15 mm thick, glabrous, longitudinally striate, red to maroon, indehiscent, stipe to 10 mm long, the apex narrowing to a spinelike beak 10–30 mm long. Flowering December–April.

Distribution. Native on sand dunes and in dry places near the coast in Tamaulipas, San Luis Potosí, and Veracruz. Naturalized elsewhere in southern Mexico.

Representative specimens. MEXICO. **Colima:** 2 km S of Rancho Blanca, *Iltis et al. 681* (WIS). **Michoacán:** a 2 km al NE de El Ranchito, 100 m, *Soto N. et al. 2794* (MEX). **Oaxaca:** 13 mi. E of Pinotepa Nacional on Mexican hwy. 200, *Hansen et al. 1531* (WIS). **San Luis Potosí:** 10.3 mi. NE of Ciudad Valles on hwy. 110, *Janzen 1923* (F). **Tamaulipas:** vicinity of Tampico, 15 m, *Palmer 133* (F, MO, NY, US). **Veracruz:** 5.1 mi. SE of Jalapa on hwy. 140, *Janzen 1921* (F, MEX, NY).

It is very possible that *Acacia sphaerocephala* represents a dry-land derivative of the widespread *A. cornigera* (Janzen, 1974). These two taxa are closely related, and with sterile material the only reliable diagnostic characteristic is the lack of secondary venation in the leaflets of *A. sphaerocephala*. With fertile material, the subglobose inflorescence less than 2× longer than wide, the circular apex of the peltate floral bracts, and the longitudinally striate legumes separate this species from *A. cornigera*.

Native populations of *Acacia sphaerocephala* are restricted to a relatively small area along the east coast of Mexico from the southern part of the state of Tamaulipas to just south of the city of Veracruz. Naturalized populations, probably resulting from seed dispersal by cattle and humans, have also been found in Colima, Michoacán, Morelos, and Oaxaca, and will undoubtedly be found elsewhere in Mexico where there is suitable habitat.

Janzen (1974) suggested that this species can be divided into a beach ecotype and an inland ecotype. The beach ecotype occurs on new dunes in the vicinity of the city of Veracruz, and the inland one throughout the remainder of the range of this species. The beach ecotype commonly forms dense stands of flat-topped shrubs mostly less than 1.5 m tall; the spines usually are not occupied by obligate acacia-ants, and the leaflets usually lack Beltian bodies. The inland ecotype, in contrast, occurs as scattered individuals that may reach 5 m in height; its spines are commonly inhabited by obligate acacia-ants, and its leaves produce numerous small Beltian bodies. These inland plants are morphologically very similar to *A. cornigera*, but usually grow on slightly drier sites. All specimens of both the inland and the beach ecotypes tested negative for cyanide production, as did fresh material of a population of 10 plants from the coastal dunes, just south of Veracruz (Seigler & Ebinger, 1987). During the present study, all beach populations of this species examined contained numerous acacia-ants.

Reported Hybrids

Hybrids between ant-acacia species and between ant-acacias and non-ant-acacias have been discussed by Janzen (1974) and more recently by Ebinger & Seigler (1992). Presently only three probable hybrids between ant-acacias have been reported. Hybrids between ant-acacias and non-ant-acacias are somewhat more common. Past studies indicate that at least four ant-acacia species hybridize with various species of the *Acacia macracantha* complex, including *A. macracantha* Humb. & Bonpl. ex Willd., *A. cochliacantha* Humb. & Bonpl. ex Willd., and *A. pennatula* (Schltdl. & Cham.) Benth. In addition to these hybrids, Janzen (1974) suggested that *A. globulifera* rarely may hybridize with non-ant-acacias, but did not list the non-ant-acacia parent.

Acacia collinsii × *A. hindsii*. These two species are sympatric in parts of their ranges and are sometimes found at the same sites on soils and in climates that are suitable for seasonal agriculture (Janzen, 1974). One sterile specimen was encountered that is intermediate between these two species. This specimen is similar to *A. collinsii* in having relatively large leaflets (7–11 mm long, 1.6–3.1 mm wide) with obvious secondary veins, and with less than 25 pairs of leaflets per pinna. It is similar to *A. hindsii* in that rachis glands are common at many of the pinnae nodes, the stipular spines are slightly flattened, and the petiolar glands are narrowly volcano-shaped. The rachis glands, for the most part, are broadly dome-shaped, typical of the petiolar glands of *A. collinsii*. Intermediate rachis and petiolar gland shapes also are common on this specimen and are slightly compressed volcano-shaped, but not as narrow as those of *A. hindsii*. The specimen is weakly cyanogenic, typical of many specimens of *A. hindsii*, but not of *A. collinsii*. Final determination of the status of this probable hybrid must await flowering and fruiting material (Ebinger & Seigler, 1992).

Representative specimen. MEXICO. **Guerrero:** Lok. 7, Mirador, 5 km S de la Base Naval de Icacos, *Brimer s.n.* (ILL).

Acacia cornigera × *A. sphaerocephala*. Janzen (1974) reported seeing a single plant of this hybrid on the dunes south of the city of Veracruz, Mexico. No specimen was cited, and none were located during this study. These species are similar morphologically, and it is probable that they hybridize.

Acacia chiapensis × *A. cornigera*. Janzen (1967a, 1974) suggested that some of the morphological variation of *Acacia chiapensis* is probably an expression of the introgression of *A. macracantha* and *A. cornigera* genes into the *A. chiapensis* population. He found hybrids to be common in a permanently wet site below the dam at Temascal, Río Chichicazapa, and at Arroyo Enmedio, between Temascal, Oaxaca and La Granja, Veracruz, Mexico. On a recent trip (May 1991) to Temascal both *A. cornigera* and *A. chiapensis* were common in disturbed sites, but no hybrid individuals were observed.

Acacia chiapensis × *A. pennatula*. Shrub or small tree to 5 m tall; twigs brown to dark reddish brown, puberulent. Enlarged stipular spines shiny, reddish brown, puberulent, usually terete in cross section, sometimes ridged or striate, broadly V-shaped with an angle of 160–180° (sometimes slightly reflexed), to 70 mm long, 4.0–5.5 mm wide near the base. Leaves 70–160 mm long; pinnae 14–30 pairs per leaf; rachis densely puberulent, rachis glands absent except between the terminal 1–3 pinna pairs; petioles grooved, densely puberulent, 4–8 mm long. Petiolar glands 1–3, nearly circular and with a depressed apex, puberulent, striate, apex 0.9–1.5 mm across. Leaflets 28–36 pairs per pinna, lightly puberulent, linear, 1.9–2.8 mm long, 0.5–0.7 mm wide, one vein from the base, lateral veins not obvious, margins ciliate. Flowers and fruits not observed.

Representative specimen. MEXICO. **Oaxaca:** edge of road near large dam at Temascal, in pasture near degraded evergreen forest, *Seigler et al. 13544* (EIU, ILL).

Janzen (1974) observed that at two sites (below the dam at Temascal, Río Chichicazapa, and at Arroyo Enmedio, between Temascal, Oaxaca and La Granja, Veracruz, Mexico) *Acacia chiapensis* may hybridize with non-ant-acacias such as *A. macracantha*. At this site, numerous plants occurred that were intermediate in spine, leaf, and branching morphology between these two species. During the present study no hybrids involving *A. chiapensis* and *A. macracantha* were found; however, on a recent trip to Temascal (May 1991) the authors collected hybrid specimens of *A. chiapensis* × *A. pennatula*. Both parents were common in the pasture where the hybrid was collected and, except for *A. cornigera*, were the only acacias in the area.

Acacia collinsii × *A. pennatula*. Shrub or small tree to 5 m tall; twigs reddish brown, glabrous to lightly puberulent. Enlarged stipular spines shiny, dark brown to black, glabrous to lightly puberulent, terete in cross section, usually abruptly narrowing at the base, symmetrical, straight, V-shaped with an angle of 80–160°, to 40 mm long, 3–5.5 mm wide near the base, some of the spines not enlarged, puberulent at least near the base, less than 6 mm long. Leaves 80–160 mm long; pinnae 13–30 pairs per leaf, 25–45 mm long, 5–9 mm between pinna pairs; rachis lightly puberulent, rachis glands absent; petiole grooved, puberulent, 8–10 mm long. Petiolar glands 2–4, scattered along the petiole, broadly dome-shaped, the apex depressed, puberulent, striate, apex 1.4–2.5 mm across, sometimes the glands overlapping and continuous to 4.5 mm long. Leaflets 23–32 pairs per pinna, usually glabrous, linear, 4.5–5.5 mm long, 0.9–1.2 mm wide, one vein from the base, lateral veins sometimes obvious. Inflorescence a densely flowered cylindrical spike, 10–14 mm long, 6–7 mm wide, in

clusters of 2–6 in the axil of slightly reduced leaves; peduncles densely puberulent, to 35 mm long; involucre usually located just below the spike, puberulent, usually 4-lobed, Floral bracts spatulate to nearly peltate with a circular apex. Corolla yellowish, about ⅔ as long as the calyx. Fruit straight, elliptical in cross section, to 80 mm long, 13–16 mm wide, glabrous, not striate, dark brown, probably dehiscing along one suture, short-stalked, base broadly cuneate, apex narrowing to a short beak.

Representative specimens. GUATEMALA. **Guatemala:** 16.2 mi. NE of Guatemala City on rd. to Puerto Barrios, *Janzen 744* (EIU, ILL). MEXICO. **Chiapas:** El Chorreadero, 5.6 mi. E of Chiapa de Corzo along Mexican hwy. 190, 2500 ft., *Breedlove 9638* (F, US); 8.5 mi. S of La Trinitaria on hwy. 190, *Janzen 571* (EIU, ILL). **Oaxaca:** 50 mi. E of Tehuantepec on hwy. 190, *Seigler & Holstein 9782* (ILL). NICARAGUA. **Estelí:** 15.8 mi. W of Sebaco, 550 m, *Janzen 742* (EIU, ILL).

The puberulent stipular spines, petioles, rachises, peduncles, and bracts of these hybrids strongly suggest that *A. pennatula* is the non-ant-acacia parent. Also, the young leaves are densely yellowish puberulent, a characteristic of young leaves of *A. pennatula*. The cylindrical spikes, the enlarged stipular spines, the presence of Beltian bodies on the lower 1–2 leaflets of most pinna pairs, the broadly dome-shaped petiolar glands, and the absence of rachis glands on the hybrid specimens indicate *A. collinsii* as the most probable ant-acacia parent.

Acacia cornigera? × *A. pennatula:* Small shrub to 2 m tall; twigs reddish brown, puberulent. Enlarged stipular spines light grayish brown, puberulent, terete to oval in cross section, symmetrical, straight, V-shaped with an angle of 80–160°, to 30 mm long, 3–4 mm wide near the base, most spines not enlarged, puberulent, less than 4 mm long. Leaves 70–120 mm long; pinnae 12–23 pairs per leaf, 22–34 mm long, 2–5 mm between pinna pairs; rachis puberulent, a nearly circular columnar gland with a depressed apex located near the node between each pinna pair; petiole grooved, puberulent, 4–7 mm long. Petiolar glands columnar, the apex depressed, usually 1 just below the first pinna pair, puberulent, lightly striate, apex 0.8–1.2 mm long. Leaflets 15–26 pairs per pinna, lightly puberulent, ciliate, linear, 2.2–4.5 mm long, 0.6–1.3 mm wide, one vein from the base, lateral veins usually not obvious. Flowers and fruits not observed.

Representative specimen. MEXICO. **Veracruz:** 24.3 mi. from Veracruz on hwy. 140, *Seigler et al. 12224* (ILL).

The wide-ranging ant-acacia *Acacia cornigera* may rarely hybridize with the non-ant-acacia *A. pennatula*. The only specimen seen of this probable hybrid was a 2-m-tall sapling collected in a pasture where both parent species were common. The only other acacia species present was *Acacia macracantha*. The dense pubescence of this hybrid, however, suggests that *A. pennatula* is the non-ant-acacia parent. Not only are the twigs and spines densely puberulent, but the leaflets are lightly puberulent and ciliate, while the columnar rachis glands with a depressed apex are similar to those found in *A. pennatula*. The enlarged spines, the presence of Beltian bodies on the lower 1–2 leaflets of some pinnae, and the well-developed rachis and petiolar glands indicate a relationship to an ant-acacia species. Because *A. cornigera* is the only ant-acacia known to occur in this part of Mexico, it is probably the ant-acacia parent. However, the relatively small leaflets generally lack secondary veins, and the petiolar glands differ from the canoe-shaped glands of typical *A. cornigera*. Final determination must await flowering and fruiting material (Ebinger & Seigler, 1992).

Acacia hindsii × *A. cochliacantha.*

Acacia ×gladiata Saff., J. Wash. Acad. Sci. 5: 359. 1915. *Myrmecodendron gladiatum* (Saff.) Britton & Rose, N. Amer. Fl. 23: 92. 1928. TYPE: Mexico. Sinaloa: vicinity of Rosario, 1849, *J. Gregg 1135* (holotype, MO; photo, F, NY; fragment and photo, US).

Shrub or small tree to 4 m tall, twigs dark reddish brown, nearly glabrous. Stipular spines light brown to reddish brown, glabrous, flattened (sometimes oval in cross section), symmetrical, widely spreading with an angle of 160 to 180°, linear-lanceolate, constricted toward the base, to 60 mm long, 5–13 mm wide. Leaves 70–130 mm long; pinnae 8–16 pairs per leaf, 15–35 mm long, 5–10 mm between pinna pairs; rachis puberulent, a columnar gland with a depressed apex located near the node between each pinna pair (sometimes absent); petiole grooved, puberulent, 7–10 mm long. Petiolar glands columnar to volcano-shaped, 1–2 scattered along the petiole, glabrous, striate, apex 0.4–1.5 mm across. Leaflets 14–20 pairs per pinna, glabrous, linear, 2.5–4.5 mm long, 0.7–1.2 mm wide, one vein from the base, lateral veins not obvious. Inflorescence a loosely flowered, cylindrical spike, 8–17 mm long, 3.5–5 mm thick, in clusters of 2–6; peduncles to 13 mm long, puberulent; involucre located at or above the middle of the peduncle, 4-lobed, puberulent. Floral bracts spatulate. Flow-

ers sessile; calyx 0.6–0.9 mm long, the lobes puberulent; corolla puberulent, maroon, 5–6-lobed, about twice as long as the calyx. Fruits not seen.

Representative specimens. MEXICO. **Oaxaca:** 3 mi. N of Puerto Escondido, *Seigler et al. 11566 (ILL)*. **Sinaloa:** 14.4 mi. NW of Rosario on hwy. 15, *Janzen 1720* (EIU, ILL), *Ortega 4884* (US).

The narrow cylindrical inflorescences, the small leaflets, and the presence of rachis glands indicate that *A. hindsii* is the ant-acacia parent, while the enlarged, flattened stipular spines suggest that *A. cochliacantha* is the non-ant-acacia parent. In many of its characteristics this hybrid is intermediate between the parents. The large, compressed stipular spines are similar to those of *A. cochliacantha*, although these spines are not spoon-shaped as in typical *A. cochliacantha*. No specimens were found on which the spines exhibited the characteristic entrance holes made by acacia-ants. Overall, the leaves are similar to those of *A. cochliacantha* in that they are relatively short and narrow. The leaflets, however, are similar to those of *A. hindsii*, being 3.0–4.5 mm long and 0.7–1.2 mm wide. In *A. cochliacantha*, in contrast, the leaflets are typically 0.8–2.4 mm long and 0.3–0.6 mm wide (Seigler & Ebinger, 1988). Furthermore, the presence of rachis glands indicates a relationship to *A. hindsii*, as does the presence of Beltian bodies on the lower 1–4 leaflet pairs of most pinnae.

Acacia hindsii × *A. pennatula.*

Acacia ×standleyi Saff., J. Wash. Acad. Sci. 4: 367. 1914. *Myrmecodendron standleyi* (Saff.) Britton & Rose, N. Amer. Fl. 23: 92. 1928. TYPE: Mexico. Nayarit: along the river in the vicinity of Acaponeta, Territory of Tepic, W Mexico, 11 Apr. 1910, *J. N. Rose, P. C. Standley & P. G. Russell 14374* (holotype, US; photo, F).

Acacia ×hirtipes Saff., J. Wash. Acad. Sci. 4: 367. 1914. *Myrmecodendron hirtipes* (Saff.) Britton & Rose, N. Amer. Fl. 23: 92. 1918. TYPE: Guatemala. Santa Rosa: along the Río de las Cañas, 3000 ft., Apr. 1892, *Heyde & Lux 3299b* (holotype, US; photo, NY).

Myrmecodendron oaxacanum Britton & Rose, N. Amer. Fl. 23: 92. 1928. TYPE: Mexico. Oaxaca: Fonameca, 110 m, 3 July 1925, *E. Makrinius 488* (holotype, US).

Shrub or small tree to 10 m tall; twigs reddish brown, glabrous to lightly puberulent. Enlarged stipular spines shiny, light gray to black, glabrous to lightly puberulent, terete to oval in cross section, the base usually flattened, symmetrical, straight, V-shaped with an angle of 80–180°, to 40 mm long, 3–7 mm wide near the base, some of the spines not enlarged, puberulent at least at the base, less than 5 mm long. Leaves 90–150 mm long; pinnae 16–31 pairs per leaf, 20–46 mm long, 4–7 mm between pinna pairs; rachis puberulent, a columnar gland with a depressed apex located near the node between each pinna pair (rarely some absent); petiole grooved, puberulent, 6–11 mm long. Petiole glands solitary, columnar to elongated, the apex depressed, puberulent, apex 1–2 mm across. Leaflets 26–40 pairs per pinna, glabrous to lightly puberulent, ciliate, linear, 2.4–3.8 (rarely 5.0) mm long, 0.7–1.1 mm wide, one vein from the base, lateral veins not obvious. Inflorescence a densely flowered cylindrical spike, 13–20 mm long, 6–8 mm thick, slightly thicker near the apex, in clusters of 1–8 in the axis of slightly reduced leaves; peduncle densely puberulent, 10–27 mm long; involucre located near the middle of the peduncle, puberulent, usually 4-lobed. Floral bracts spatulate. Corolla yellowish to reddish, about twice as long as the yellowish calyx. Fruit straight, elliptical in cross section, 40–100 mm long, 8–12 mm wide, glabrous, not striate, black to dark brown, dehiscent along one suture, short stalked, base broadly cuneate, apex narrowing to a short beak.

Representative specimens. GUATEMALA. **Escuintla:** in pasture above Palín, 1500 m, *Standley 60100* (F). **Guatemala:** 19 km S of Guatemala City on C. A. 8, *Janzen 764* (EIU, ILL). HONDURAS. **Comayagua:** tree in dry gulch near San Luís, close to the river, *Hazlett 1445* (MO). MEXICO. **Chiapas:** a unos 3 km de Ocosingo, por la orilla de la carretera que va a Tonina, *Shapiro & Elliott 471* (MICH, MO); 3.9 mi. NE of Arriaga, *Janzen 758* (EIU, ILL). **Jalisco:** Reserva Biosfera de la Sierra de Manantlán, 16 km by new dirt road WSW of El Terrero, *Cochrane et al. 11730* (WIS). **Nayarit:** 16 mi. E of San Blas, *Johnson 109-73* (MO). **Oaxaca:** Nejapa, a 5 km al S–SW de Santa Maria Zacatepec, *Sousa et al. 10587* (MEX); 46.7 mi. W of Tehuantepec, along hwy. 190, *Janzen 748* (EIU, ILL).

The densely puberulent stipular spines, petioles, rachises, peduncles, and bracts are similar to those found in *A. pennatula*. Also, the spatulate floral bracts are typical of *A. pennatula*, as are the columnar petiolar glands with a depressed apex. These hybrid specimens also have numerous characteristics of *A. hindsii*. The presence of enlarged stipular spines, Beltian bodies on the lower 1–2 leaflets of most pinnae, the well-developed rachis glands, and the elongated spikelike inflorescence all indicate a relationship to *A. hindsii*. Considering the distribution of this hybrid, the only other possible ant-acacia parent is *A. collinsii*. The lack of obvious secondary veins in the leaflets, the presence of rachis glands, and the small size of the leaflets, however, indicate that *A. collinsii* is not the ant-

acacia parent. Of the specimens of this hybrid tested for cyanide production, four gave a weak to moderate positive reaction.

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NOTES ON *ARISTEA* AITON (IRIDACEAE: NIVENIOIDEAE): TAXONOMY, CHROMOSOME CYTOLOGY, AND PHYLOGENY

Peter Goldblatt[1]

Abstract

Capsules with long pedicels, a thicker stem, wider leaves, and inflorescences borne on long leafless scapes confirm the current taxonomy that maintains *Aristea alata* separate from *A. abyssinica* and *A. cognata*. The two latter, however, are difficult to distinguish. Although some southern African plants referred to *A. cognata* are smaller than tropical African *A. abyssinica*, variation within and between populations suggests that smaller individuals are depauperate, due to local edaphic or seasonal conditions. I conclude that *A. cognata* and *A. abyssinica* are best treated as conspecific, and I do not recommend recognition of infraspecific taxa. Section *Ancipites*, to which these species belong, is probably heterogeneous, and the removal of *A. nitida* to section *Cladocarpae* is recommended. Three species of section *Pseudaristea* should also be added to section *Cladocarpae*. Lectotypes, lacking for sections *Ancipites* and *Pseudaristea*, are designated here. New collections of southern African *A. singularis*, only species of section *Singulares*, change our understanding of the species. It is probably not primitive in the genus, but appears specialized in growth form, inflorescence structure, seed morphology, and number of seeds per locule. The species is most likely related to, and perhaps nested within, section *Aristea* (= section *Cyaneae* Weim.). Original chromosome counts for seven species, five not before counted, confirm a base number of $x = 16$ for *Aristea*.

Aristea Aiton, the largest genus of Nivenioideae, one of four subfamilies of Iridaceae currently recognized (Goldblatt, 1990), comprises some 50 species of sub-Saharan Africa and Madagascar (Weimarck, 1940). An estimated 44 species of *Aristea* occur in Africa, of which 40 are found in southern Africa, and at least 8 occur in tropical Africa. Five of the tropical African species are shared with southern Africa, and three are endemic. The six species that occur in Madagascar are endemic to the island (Goldblatt, 1991). The systematics of the genus is fairly well understood (Weimarck, 1940; Vincent, 1985; Goldblatt, 1991), but some aspects of the taxonomy of the genus in Africa require attention.

In particular, the circumscription of taxa of the tropical and southern African *Aristea abyssinica*–*A. alata*.–*A. cognata* complex is confused, and section *Ancipites* Weim., to which they belong, is probably heterogeneous. I suggest the removal of the Madagascan *A. nitida* Weim. from section *Ancipites* to section *Cladocarpae* Weim. *Aristea nitida* resembles closely *A. cladocarpa* Baker, also Madagascan, the type and currently only species of this section. I also recommend the inclusion of three species of section *Pseudaristea* Pax, *A. ecklonii*, *A. ensifolia*, and *A. pusilla*, in section *Cladocarpae*. This leaves section *Pseudaristea*, which is lectotypified here, more narrowly circumscribed and exclusively southern African. Section *Ancipites* as understood here is also morphologically uniform as regards its defining features and is evidently monophyletic. The expanded section *Cladocarpae* appears morphologically coherent, despite the addition of both African and Madagascan species.

In southern Africa, new collections of *Aristea singularis* Weim., thought by Weimarck (1940) to be the most primitive species of the genus, and only member of section *Singulares* Weim., indicate a need for a reappraisal of its relationships. *Aristea singularis* is probably most closely related to species of section *Aristea* (= section *Cyaneae* Weim.), one of the more specialized of the eight sections of the genus recognized by Weimarck (1940). Original chromosome counts, made by M. Takei, Biological Institute, Oita University, Japan, are also reported here for seven species of *Aristea*, five of which have not before been counted. The new counts include the first cytological reports for the Madagascan members of the genus.

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1. *Aristea abyssinica* and *A. cognata* and Associated Sections

Species of section *Ancipites* are apomorphic in *Aristea* in having a semi-scapose habit. The flowering stem, surrounded by a basal cluster of leaves, consists of a single long internode terminating in one or two apical inflorescences subtended by a short subterminal leaf. In addition the stem is strongly compressed and 2-winged (ancipitous according to Weimarck), also a derived condition. The section was treated by Weimarck (1940) as comprising *A. anceps* Eckl. ex Klatt, *A. alata* Baker, *A. alata* subsp. *abyssinica* (Pax) Weim. (based on *A. abyssinica* Pax), *A. alata* subsp. *bequaertii* (De Wild.) Weim. (based on *A. bequaertii* De Wild.), *A. goetzei* Harms, *A. cognata* N. E. Br. ex Weim., and *A. nitida* Weim. A few additional named species were regarded by Weimarck as conspecific with *A. alata* and need not be discussed further. Marais (1987) has already pointed out problems in Weimarck's treatment of *A. abyssinica* as a subspecies of *A. alata*. Marais considered *A. abyssinica* as resembling more closely the largely southern African *A. cognata* than the East African *A. alata* subsp. *alata*, and he regarded *A. abyssinica* and *A. cognata* as conspecific but separable at subspecific rank (his error in choosing to use the later synonym *A. cognata* for the species was corrected by Goldblatt (1993a)).

Aristea alata is readily distinguished from the remaining species (and subspecies) of section *Ancipites* by its more robust appearance, with stems 4–5 mm wide and leaves usually 4–5 mm wide. The terminal flower cluster is usually borne some distance above the subterminal (and only) cauline leaf, and the rhipidial spathes and bracts are reddish brown and 12–15 mm long (rarely almost 20 mm). The capsules are 9–12 mm long, and borne on long pedicels normally 5–12 mm long.

Plants of *Aristea abyssinica* sensu Marais are less robust, have stems 1–4 mm wide and leaves 1.3–3(–4) mm wide, and the terminal flower cluster is often nearly sessile or borne close to the cauline leaf. The capsules are 5–8 mm long and subsessile (the pedicels usually less than 2 mm long). The rhipidia have spathes 6–9 mm long, and the floral bracts are about the same length. Both the spathes and the bracts are scarious and have the median part reddish brown and the edges nearly transparent, and evidently readily torn, for they are often somewhat lacerated in plants collected late in the season. As noted by Goldblatt (1993a), subspecies *abyssinica* and subspecies *cognata* are not easily distinguished, and at least provisionally I preferred not to recognize them as separate subspecies. Marais regarded subspecies *cognata* as restricted to southern Africa and Zimbabwe, and subspecies *abyssinica* as extending from Zambia and Malawi northward into Kenya and Ethiopia (where *A. alata* also occurs), but he did not enumerate their differences.

I have examined plants from across the entire range of *Aristea abyssinica* and find only weak support for maintaining *A. cognata* as separate at any taxonomic level. Consistent differences, if indeed any exist, between southern and tropical African members of the species are not evident in the mass of herbarium material available, much of it lacking well-preserved flowers. Floral differences, if there are any, remain to be demonstrated. Plants of *A. abyssinica* (including the type) in tropical Africa are 15–40 cm high, have capsules 6–8 mm long, and the leaves are about half, less often two-thirds, as long as the stems and 3–4 mm wide. In southern Africa, plants Marais regarded as subspecies *cognata* are 8–35 cm high, have stems 1–2 mm wide, capsules 5–8 mm long, and in shorter plants the leaves are as long as the stems. The leaves are also often narrower than in plants from tropical Africa, 1.3–3 mm wide.

The differences fail to separate specimens from the two areas consistently. Even in the same collection southern African plants may range from 8 to 35 cm high, and taller plants in general have stems exceeding the relatively broader leaves. Dwarfed individuals are probably depauperate due to edaphic or environmental conditions. For example, in the plants growing along paths where there is only low, trampled ground cover, *A. abyssinica* is usually 8–12 cm high, while plants nearby growing among taller grasses are 25–30 cm high. Weimarck's (1940) key to *A. cognata* and *A. alata* subsp. *abyssinica* also demonstrates the problem of overlapping vegetative characters. He separated *A. cognata* from subspecies *abyssinica* on the basis of a stem 1–1.8 mm wide (actually they may be up to 2 mm wide) vs. 2–5 mm wide, and capsules 5–8 mm long vs. 6–9 mm long. Additionally, Weimarck regarded the flowers as differing in size, those of *A. cognata* having tepals 2.8–3.5 mm wide vs. 6–7.5 mm in subspecies *abyssinica*. This reflects poor preservation of the material available to him. In fact, the tepals of plants he would have referred to *A. cognata* are 4.5–6.5 mm wide, while those of plants from tropical Africa are 4–7 mm wide.

Strictly southern African *Aristea anceps* is readily separated from *A. abyssinica* by its substantially longer rhipidia, 12–15 mm long, and

capsules (6–)9–12 mm long. The light rusty brown rhipidial spathes and floral bracts, sometimes green in the midline, and larger flowers than those normally found in *A. abyssinica* also help distinguish *A. anceps*.

The taxonomy of *Aristea abyssinica* is as follows:

Aristea abyssinica Pax in Engler, Hochgebirgsfl. Trop. Afr. 173. 1892. *Aristea alata* subsp. *abyssinica* (Pax) H. Weim, Acta Univ. Lund, new ser. 36(1): 44. 1940. *Aristea cognata* subsp. *abyssinica* (Pax) Marais, Kew Bull. 42: 932. 1987. TYPE: Ethiopia. Tigre and Begemder, *Schimper 279* (holotype, B not seen; isotype, K).

Aristea johnstoniana Rendle, Trans. Linn. Soc. ser. 2, Bot. 4: 48. 1895. TYPES: Malawi. Mt. Mulanje, June 1891, *Whyte 14* & *81* (syntypes, BM, K).

Aristea bequaertii De Wild., Feddes Repert. Sp. Nov. Regni Veg. 11: 509. 1913. *Aristea alata* subsp. *bequaertii* (De Wild.) H. Weim., Acta Univ. Lund, new ser. 36(1): 46. 1940. TYPE: Zaire. Shaba, Lubumbashi (Elisabethville), 3 Apr. 1912, *Bequaert 303* (lectotype, designated by Weimarck (1940), BR not seen).

Aristea homblei De Wild., Feddes Repert. Sp. Nov. Regni Veg 11: 509. 1912. TYPE: Zaire. Shaba. Welgelegen, in 1912, *Corbusier sub Homblé 615* (holotype, BR not seen).

Aristea cognata N. E. Br. ex H. Weim., Acta Univ. Lund, new ser. 36(1): 39. 1940. TYPE: South Africa. Transvaal, Houtbosch, 1875–1880, *Rehmann 5769* (holotype, K).

I have not been able to examine the types of either *Aristea tayloreana* or *A. goetzei*. The former was included by Weimarck in *A. alata* subsp. *abyssinica*, and the latter was maintained as a separate species. From the description this plant, restricted to the Lukwangule Plateau in the Uluguru Mountains of eastern Tanzania, may be very close to typical *A. alata*.

The last species in section *Ancipites*, *Aristea nitida*, from Madagascar, was imperfectly known to Weimarck. Later collections indicate that it does not accord with the definition of section *Ancipites*. The stems bear 2–3 leaves and 3–8 flower clusters, evenly spaced along the upper part, some of them subtended by short leafy bracts. Morphology supports Goldblatt & Le Thomas (1992) in regarding *A. nitida* as most closely resembling *A. cladocarpa*, a species referred by Weimarck to a monotypic section *Cladocarpae*. The main difference between the two species is actually in the capsules, those of *A. nitida* being ovoid and nearly sessile, whereas those of *A. cladocarpa* are cylindric and shortly pedunculate. The resemblance between the two species is so strong that in the absence of capsules it is difficult to distinguish them, and separation at the sectional level seems inappropriate.

In turn, *Aristea cladocarpa* resembles fairly closely the tropical and southern African *A. ecklonii*, placed by Weimarck in section *Pseudaristea*. Like *A. cladocarpa*, *A. ecklonii* has cylindric capsules borne on short pedicels, and compressed 2-winged stems with leaves and branches along the axis. I propose modifying the current infrageneric classification by expanding section *Cladocarpae* to include *A. nitida*, *A. ecklonii*, and at least *A. pusilla* and *A. ensifolia*; the two latter are currently assigned to section *Pseudaristea* and evidently closely allied to *A. ecklonii*. Both sections *Cladocarpae* and *Ancipites* appear to be closely allied to section *Eucapsulares* Goldblatt (= section *Euaristea* Weim.), a fairly large and diverse assemblage including both African and Madagascan species. Further study is needed to determine their mutual relationships, including the possibility that sections *Cladocarpae* or *Ancipites* are nested within *Eucapsulares*.

Section *Pseudaristea* is consequently circumscribed more narrowly to include only those species with apomorphic cylindric, deeply 3-lobed capsules of a more or less woody texture. These capsules are also derived in *Aristea* in being tardily dehiscent, possibly serotinous. Species of the section also have unusually large flowers for the genus and relatively long anthers (Weimarck, 1940), both of which are probably apomorphic states, as are the distinctive capsules. When described by Pax (1888) section *Pseudaristea* included three species, only two of which were specifically named, *A. pusilla* and *A. spiralis*. I designate the latter as lectotype for the section because it is closer to the definition of the section than the poorly understood *A. pusilla* (neither Pax nor Weimarck indicated types for the sections they described). Section *Pseudaristea* as redefined includes only four western southern African species. In addition to *A. spiralis* these are *A. biflora* Weim., *A. lugens* (L.f.) Weim., and *A. simplex* Weim. I am uncertain of the affinities of the two remaining members of section *Pseudaristea* sensu Weimarck: both *A. zeyheri* Baker and *A. pauciflora* Wolley-Dod should provisionally remain in section *Cladocarpae* until and unless they can be placed elsewhere with more certainty. Their floral and capsular features are poorly known, and do not appear to accord with section *Pseudaristea* as redefined here. Lectotypes, not previously selected, are designated here for sections *Ancipites* and *Pseudaristea* as follows:

Aristea section **Ancipites** Weim., Acta Univ. Lund new ser. 36(1): 36. 1940. TYPE: *A. anceps* Ecklon ex Klatt (lectotype, here designated).

The first species of the section to be discovered, *A. anceps* also accords unambiguously with the characters of the section and there seems no doubt or confusion about its status or circumscription.

Aristea section **Pseudaristea** Pax, Nat. Pflanzenf. edition 1, 2(5): 153. 1888. TYPE: *A. spiralis* (L.f.) Ker Gawl. (lectotype, here designated).

2. *ARISTEA SINGULARIS* WEIMARCK

Aristea singularis, until now poorly understood, is a rare endemic of the northern Cedarberg Mountains in the northwestern Cape, South Africa. It was regarded by Weimarck (1940) as the sole species of his section *Singulares*. He interpreted it as one of the most unspecialized species in the genus and used this as justification for placing it at the beginning of his treatment, before three fairly generalized sections, mostly of tropical Africa and Madagascar. Study of new collections of *Aristea singularis* suggests that its true affinities probably lie with the specialized, predominantly southwestern Cape section *Aristea* (= section *Cyaneae* Pax).

The type and only collection of *Aristea singularis* known to Weimarck in 1940 when he described the species is from Boontjiesrivier, at the northern end of the Cedarberg (*Leipoldt s.n.* BOL 18888). Additional collections have now been made near Pakhuis Pass, both near the top of the Pass and along the track to Heuningvlei (*Barker 10453 NBG, Taylor 11001 NBG, Goldblatt 7253A MO*). Plants appear to be relatively uncommon and occur in fairly sheltered sites in the shade of rocks, but not in close proximity to streams or pools. The type collection is thought to have been made along a stream bank, which may explain its particularly robust and foliose appearance compared to all later collections (Weimarck, 1940: tab. 1).

Vegetative form. The most striking feature of *Aristea singularis* is the aerial stolons that give the species its characteristic appearance. The stolons consist of long, fairly thick and hard (but without secondary thickening) internodes produced from the base of the parent plant, which consists of a small dense tuft of lanceolate, equitant leaves and a number of short flowering stems. The ends of the stolons bear clusters of short leaves and under suitable conditions roots are produced, anchoring the stolons some distance from the parent plant. The stolons persist for several years so that discrete tufts of leaves and flowering stems remain attached to one another in mature individuals. *Aristea singularis* is thus neither normally an erect nor a weakly climbing plant, as Weimarck supposed.

This growth form is not common in *Aristea* nor in other Iridaceae, but it is not significantly different from that in a few other species of the genus. *Aristea dichotoma* (section *Aristea*) has aerial and branched rhizomes that may root a short distance from the parent plant, and subsequently give rise to a tuft of leaves. Elongate rhizome-like underground stolons are also produced by some other species of section *Aristea*, including *A. africana* and *A. glauca*, both of which may form sparse or even dense mats of what seem to be several plants, all connected by rhizomes just below the soil surface. This is presumably comparable and homologous with the condition in *A. singularis* and *A. dichotoma*, which have aboveground stolons or rhizomes.

Inflorescence and flowers. The flowering stems of *Aristea singularis* are relatively short and generally do not exceed the leaves. They are laxly branched and each branch arches outward, terminating in an inflorescence of two, or occasionally one, pendulous flower, the pedicel of which is subtended by opposed coriaceous bractlike structures. Like other species of *Aristea*, the flowers are blue and consist of 6 subequal tepals, which in *A. singularis* are united basally for ca. 2 mm. The stamens are short, straight, and symmetrically disposed. The style reaches only to mid anther level and seems hardly, if at all, lobed at the apex. The pendent flower is unique in *Aristea*, but is otherwise unremarkable. The inflorescence is either a binate rhipidium, with each of the two rhipidial units bearing a single flower, or the whole inflorescence consists of a single flower, subtended by two bractlike structures. The latter is remarkable, for inflorescence units with one flower are rare elsewhere in *Aristea*, although they are known in a few species where they are the exception (Weimarck, 1940: 105–107).

A binate rhipidium is apomorphic for Nivenioideae (Goldblatt, 1990; Manning & Goldblatt, 1991) and is a structure believed to be the result of the union of two rhipidia (an inflorescence type characteristic of Iridaceae). In most species of *Aristea* the binate rhipidia have four or more flowers; in a few species, by reduction, they have only two. Two-flowered binate rhipidia are regarded as plesiomor-

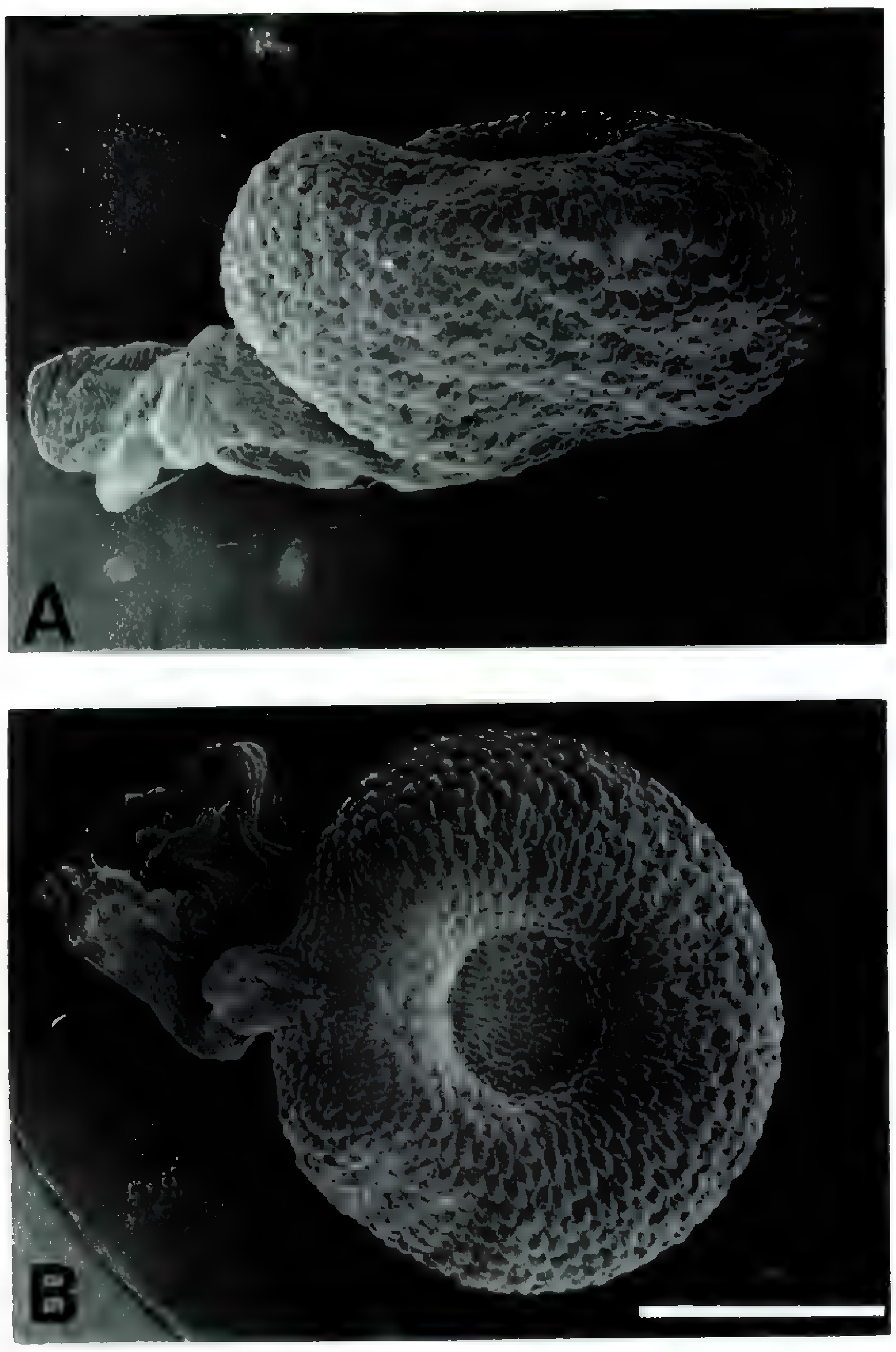

FIGURE 1. Seed morphology of *Aristea singularis*. A. Lateral view, showing the compressed-spherical shape, and funicular nature of the elaiosome.—B. Dorsal view, showing the collapsed chalazal tissue. Scale bar, 1 mm.

phic for three genera of Nivenioideae, *Nivenia*, *Klattia*, and *Witsenia* (Goldblatt, 1993b), but in some species of *Nivenia* the binate rhipidia are one-flowered. The binate nature of the inflorescence units in these species is maintained except that one of the rhipidial units is represented only by its subtending bract (spathe), which lacks a flower or floral bract. In *A. singularis* there is only one rhipidial spathe and one floral bract. The binate ancestry of the inflorescence is then not evident when the inflorescences are one-flowered.

Capsules and seeds. The pendent capsules are deeply and narrowly 3-lobed along their entire length, an apomorphic condition very closely resembling the narrowly winged capsules of sections *Aristea* and *Racemosae*. Unlike the radially compressed seeds of these two sections, the seeds of *A. singularis* are radially compressed spheres, on the chalazal side of which there is a deep invagination (Fig. 1A, B). The seeds are not numerous, as Weimarck (1940) believed, based on his examination of immature capsules. There are normally two seeds per locule or, apparently by reduction, only one. The seeds are also remarkable in having a large cream to yellowish elaiosome-like appendage, unique in the genus (Fig. 1A, B). The elaiosome appears to be an elaboration of the funicle. Both the capsules and the seeds of *A. singularis* are most likely derived in *Aristea*, and suggest a need for a reevaluation of its systematic position.

Probable relationships. The relatively short flowering stems of *Aristea singularis*, held not much above the level of the leaf apices, are similar to those of section *Aristea*, although binate rhipidia with one flower per unit or a solitary flower are not. Species of section *Aristea* typically have binate rhipidia with two flowers, one to each of the subunits. Weimarck, however, interpreted the single-flowered rhipidia as plesiomorphic because they resemble one flower and its associated bracts in the spikes of Iridaceae subfamily Ixioideae (individual flowers borne on a vertical axis, subtended by an outer and opposed inner bract). I disagree with this interpretation. Binate rhipidia are more likely to be the result of fusion of two multiflowered rhipidia, themselves the plesiomorphic inflorescence type in Iridaceae or at least in subfamilies Nivenioideae and Iridoideae (Goldblatt, 1990). The reduction in number of flowers per rhipidial subunit seems most likely to be apomorphic. Thus, the apomorphic 2- or 1-flowered binate rhipidia in *A. singularis* are consistent with its other specialized features, the apomorphic growth form, reduced number of seeds, strongly lobed capsules, and presence of an elaiosome on the seed.

Aristea singularis seems to be a relatively specialized member of *Aristea*, derived in several features. Its relationships probably lie with species of section *Aristea*. Except for the narrowly 3-lobed capsules (rather than 3-winged), rounded seeds (rather than radially flattened), and well-developed aerial stolons, it falls within the section. These discordant features are all apomorphic and reveal nothing about its relationships. The rounded seeds, in particular, may be a secondary condition associated with the presence of an elaiosome and a novel mode of seed dispersal, presumably by ants. Whether section *Singularis* still merits recognition is debatable, but it is certainly not basal in the genus. It is more likely nested within section *Aristea*, and its continued recognition would render that section paraphyletic. A decision about its sectional taxonomy must wait for a more detailed phylogenetic analysis of *Aristea*.

3. Chromosome Cytology

Using a squash technique on root tips of germinating seedlings, fully explained elsewhere (Goldblatt et al., 1993), diploid numbers were established

TABLE 1. New chromosome numbers in *Aristea* with collection data. Vouchers are at the Missouri Botanical Garden (MO).

Species	Diploid number $2n$	Collection data
Section *Ancipites*		
A. abyssinica Pax	64	Zimbabwe, Nyanga, *Goldblatt 9070*
	32	S. Africa, near East London, *Goldblatt & Manning 9532*
A. anceps Ecklon ex Klatt	32	S. Africa, near Fish River mouth, *Goldblatt 9053*
Section *Eucapsularis* (= section *Euaristea* Pax)		
A. angolensis Baker	32	Malawi, Zomba Mt., *Goldblatt 4528*
A. angustifolia Baker	32	Madagascar, Andringitra, *Goldblatt & Schatz 8971*
A. kitchingii Baker	32	Madagascar, source unknown, no voucher
A. schizolaena Harvey	32 + 2B	S. Africa, Cape, near East London, *Goldblatt & Manning 9531*
Section *Racemosae*		
A. juncifolia Baker	32	S. Africa, Cape Point Reserve, *Goldblatt 9056*

by M. Takei for seven species of *Aristea*, five of these not before counted (Table 1). Results confirm earlier observations (Goldblatt, 1971) that $x = 16$ is the basic chromosome number in *Aristea*. Six of the species examined, including one from tropical Africa and two from Madagascar, are diploid, $2n = 32$, and the seventh, *A. abyssinica*, has both diploid and tetraploid, $2n = 64$, populations. Additional counts in this widespread African species may be helpful in understanding its systematics. Two other species of the genus, the tropical and eastern southern African *A. ecklonii* and the Cape *A. glauca*, are also reported to have both diploid and tetraploid populations (Goldblatt, 1971). Plants of *A. schizolaena* examined actually had $2n = 34$, but consistently had two small darkly staining chromosomes at mitotic prophase, and it seems likely that these represent B chromosomes. I assume that the correct way to view this karyotype is to record it as $2n = 32 + 2B$.

Chromosomes of *Aristea* are relatively small, 0.9–2.3 μm long, and meta- to submetacentric (Fig. 2). The longest chromosome pair has large satellites 0.5–0.7 μm long in all the species examined. The chromosomes stain uniformly at late mitotic prophase, excepting for the one extra pair

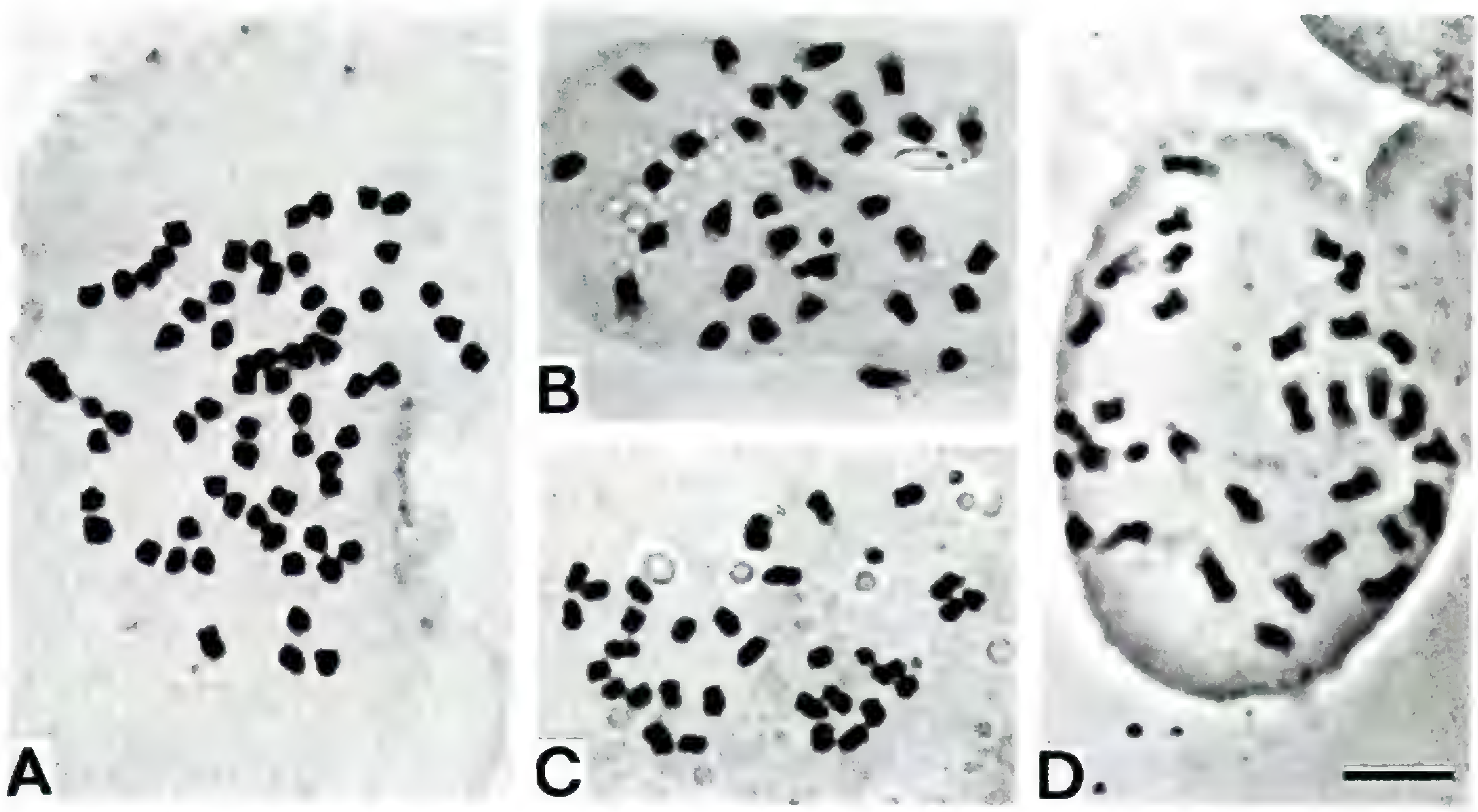

FIGURE 2. Mitotic metaphase in *Aristea*.—A. *A. abyssinica*.—B. *A. anceps*.—C. *A. angolensis*.—D. *A. angustifolia*. Scale bar, 5 μm.

in *A. schizolaena*. These chromosomes stain darkly in early prophase, for which reason they are regarded as B chromosomes. No variation was, however, noted in the number of the darkly staining chromosomes in different cells, often a feature of B chromosomes.

Aristea shares its base number of $x = 16$ with the three southern African woody genera of Nivenioideae, *Klattia*, *Witsenia*, and *Nivenia*. The remaining genera of the subfamily are the Madagascan saprophyte *Geosiris*, which is unknown cytologically, and the Australasian *Patersonia*. The ancestral base number for this genus of ca. 22 species is uncertain (Goldblatt, 1979), but may be $x = 11$ (Goldblatt & Takei, in press).

Phylogeny

Phylogeny of *Aristea* has so far received little attention, but variation in capsule and seed morphology, used by Weimarck (1940) for sectional delimitation, makes it seem likely that such a study will be rewarding. Patterns of variation in capsule and seed morphology suggest that the tropical and eastern southern African species are less specialized than those occurring in the winter-rainfall southwestern Cape Region of South Africa. The Cape species have apomorphic, narrowly 3-winged capsules of at least two types, and diverse seed morphology. Pollen grains, rather poorly sampled to date, are more diverse than in most other genera of Iridaceae (Schulze, 1971; Goldblatt & Le Thomas, 1992). Additional palynological observations will be valuable in establishing phylogenetic trends in *Aristea*, which in turn will make it possible to understand its phytogeography better. Chromosome cytology of the genus is poorly known, although the record to date provides little incentive for further investigation. Additional counts for seven species reported here confirm the pattern already evident in the genus. All 15 species so far examined have $x = 16$; 12 species are diploid, $2n = 32$, and three more, *A. glauca*, *A. ecklonii*, and *A. abyssinica*, have both diploid and tetraploid populations, $2n = 32$ and 64.

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ALTERNATIVE GENES FOR PHYLOGENETIC RECONSTRUCTION IN PLANTS: INTRODUCTION

Pamela S. Soltis and Douglas E. Soltis[1]

The past five years have witnessed perhaps unparalleled enthusiasm for new approaches in plant systematics. Although systematics often is, and should be, viewed as an "unending synthesis" (Constance, 1964), incorporating data from new sources as they become available, it is our impression that few approaches have been embraced as heartily by the community of plant systematists as have DNA sequencing and phylogenetic analysis. The ease with which segments of DNA can be amplified and sequenced, coupled with sophisticated computer programs for analysis of data (e.g., PAUP and PHYLIP), have greatly improved our collective ability to retrieve plant phylogeny.

Although analysis of DNA sequences could potentially be used to resolve problems at all taxonomic levels, most studies to date have used sequences of the chloroplast gene *rbc*L and have addressed questions at the family level and above. For example, the third issue of Volume 80 of the *Annals of the Missouri Botanical Garden* was devoted to papers using *rbc*L sequences to reconstruct plant phylogeny. The large analysis of seed plants (Chase et al., 1993), complete with warts, provides a useful framework for future studies using both molecular and nonmolecular characters. In addition, the companion papers in that issue address specific taxonomic problems at the intergeneric to intraclass levels, with an emphasis on intra- and interfamilial relationships. These papers and others have demonstrated the versatility of comparative *rbc*L sequencing for phylogeny reconstruction: although rates of *rbc*L sequence evolution typically make this gene appropriate for familial and/or ordinal studies, rates vary sufficiently among groups to permit its occasional use at both lower and higher taxonomic levels.

Despite the broad taxonomic spectrum for which *rbc*L sequences can provide meaningful data, many important questions of plant phylogeny and evolution cannot be addressed using *rbc*L. For these questions, at both recent and deep levels of plant phylogeny, alternative genes to *rbc*L are needed. To resolve interspecific relationships and explore patterns and processes of speciation, more rapidly evolving genes or DNA segments are needed; in contrast, to address more rigorously the evolutionary history of all land plants, all seed plants, or even all angiosperms, a more conservative gene is required. In this issue, the contributors explore the potential of eight genes or DNA regions for phylogenetic reconstruction at an array of hierarchical levels. Both nuclear and chloroplast genes are evaluated. Three regions of the nuclear ribosomal RNA

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cistron are explored: the 18S gene (Nickrent & Soltis), the internal transcribed spacers (ITS; Baldwin et al.), and the 26S gene (Bult et al.). Small multigene families from the nuclear genome may also carry phylogenetic signal: the phytochrome gene family (Mathews et al.) and the small heat shock gene family (Waters). Three genes from the chloroplast genome are also considered: *atp*B (Hoot et al.), *ndh*F (Olmstead & Reeves), and *mat*K (Johnson & Soltis). Each paper describes the location, size, structure, and rate of evolution of the chosen gene and discusses its potential for phylogenetic study.

The first nine papers in this issue derive from a symposium entitled, "Alternative Genes in Phylogenetic Reconstruction," presented at the 1993 AIBS meetings and cosponsored by the American Society of Plant Taxonomists and the Botanical Society of America. We hope this collection of papers will stimulate the development and application of new genes for phylogeny reconstruction in plants.

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PHYLOGENETIC INFERENCE IN SAXIFRAGACEAE SENSU STRICTO AND *GILIA* (POLEMONIACEAE) USING *mat*K SEQUENCES[1]

Leigh A. Johnson and Douglas E. Soltis[2]

ABSTRACT

Comparative sequencing of the maturase-encoding chloroplast gene *mat*K has great potential for reconstructing phylogenetic relationships not only within families, but also within genera of land plants. This gene of 1550 bp is easily amplified due to highly conserved, flanking coding regions that include the *trn*K exons, *rps*16, and *psb*A. Several available sequencing primers also have wide applicability. Parsimony analysis of 45 *mat*K sequences representing Saxifragaceae sensu stricto provides a level of resolution comparable to that obtained via chloroplast DNA restriction site analysis. Furthermore, this analysis suggests relationships among genera and species that are highly concordant with the results of separate analyses of *rbc*L sequences and chloroplast DNA restriction sites, and with those of combined analyses of these three chloroplast DNA data sets. Parsimony analysis of 31 *mat*K sequences representing all six sections of *Gilia* (Polemoniaceae) and 10 allied genera provides strong evidence for the polyphyly of *Gilia* and suggests relationships among sections of *Gilia* that are highly concordant with a recent ITS sequence analysis of the Polemoniaceae. Our analyses suggest that *mat*K sequences are not strongly biased toward transitions, and the frequency of mutations at the first and second codon positions approach the frequency of mutations in the third codon position.

Investigation of the chloroplast genome, either through analysis of restriction site mutations, structural rearrangements, or DNA sequences, has dominated plant molecular systematic research during the past decade. These approaches have proven extremely useful in addressing a broad range of systematic and evolutionary questions at all levels of taxonomic hierarchy. Of these approaches, comparative sequencing of chloroplast, as well as nuclear, genes has become particularly popular in recent years, due in large part to the relative ease of generating sequences and the unambiguity of the data. The large number of recent systematic studies employing sequencing of the chloroplast gene *rbc*L attests to the enormous phylogenetic potential of comparative sequencing (e.g., Brunsfeld et al., 1994; Chase et al., 1993; Conti et al., 1993; Donoghue et al., 1992; Gadek & Quinn, 1993; Giannasi et al., 1992; Kim et al., 1992; Kron & Chase, 1993; Olmstead et al., 1993; Morgan & Soltis, 1993; Price & Palmer, 1993; Qiu et al., 1993; Rodman et al., 1993; Smith et al., 1993; Soltis et al., 1990). Although *rbc*L sequence analysis has overshadowed the use of other gene sequences in plant systematics, the phylogenetic utility of several other DNA regions, both nuclear and organellar, has been investigated in plants. Among chloroplast genes, *atpβ* (Ritland & Clegg, 1987; Hoot et al., 1995 this issue), *mat*K (Johnson & Soltis, 1994; Steele & Vilgalys, 1994), and *ndh*F (Olmstead & Sweere, 1994; Olmstead & Reeves, 1995, this issue) provide regions with demonstrated utility for inferring phylogenies at taxonomic levels unresolvable with *rbc*L sequences alone. The use of these, and other regions of chloroplast DNA (cpDNA), such as noncoding intergenic spacers (Gielly & Taberlet, 1994; Golenberg et al., 1993), for phylogenetic inference suggests that analysis of the chloroplast genome will continue to provide important information for systematics.

Among protein coding regions in the chloroplast genome, *mat*K (ORFK) is one of the most rapidly evolving (Wolfe, 1991). The chloroplast gene *mat*K

[1] We thank Paul Gadek, Christopher Quinn, Clifford Morden, and Jeff Palmer for sharing unpublished results of their studies involving *mat*K; John Wiens and Tod Reeder for providing their unpublished manuscript on "pseudofossils"; Mark Porter for providing information and discussion concerning his ITS sequence analysis of the Polemoniaceae; Robert Jansen for providing *ndh*F sequences for comparative purposes; Mark Chase, Paul Gadek, and Pam Soltis for providing helpful comments on an earlier draft of this manuscript; and the many individuals who provided plant material used for sequencing. This research was supported by NSF grants BSR 9007614 and DEB 9307000 to DES, DEB 9321788 to LAJ and DES, and grants from Sigma Xi, the American Society of Plant Taxonomists, and the California Native Plant Society to LAJ.

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is approximately 1550 base pairs (bp) in length and encodes a maturase involved in splicing type II introns from RNA transcripts (Neuhaus & Link, 1987; Wolfe et al., 1992). In all photosynthetic land plants examined to date, *mat*K is located within an intron of approximately 2600 bp located between the 5′ and 3′ exons of the transfer RNA gene for lysine, *trn*K (Fig. 1). However, in the reduced chloroplast genome of the nonphotosynthetic parasite *Epifagus virginiana* (L.) Barton (Orobanchaceae), neither the *trn*K coding regions nor associated intron are present (Wolfe et al., 1992). In *Epifagus*, *mat*K is bounded by *trn*Q and the *psb*A pseudogene. The presence of *mat*K in the very reduced plastid genome of *Epifagus* suggests that *mat*K is functionally important and has a broader intron splicing role than simply splicing the *trn*K intron in which it typically occurs (Wolfe et al., 1992). Alignment of seven complete *mat*K amino acid sequences representing bryophytes, gymnosperms, monocots, and dicots reveals the presence of more conserved regions interrupted by stretches of sequence having little similarity (C. Morden and J. Palmer, pers. comm.).

We recently reported that *mat*K sequences of only 750 bp provided resolution of relationships in Saxifragaceae sensu stricto (s. s.) comparable to that obtained via restriction site analysis of the entire chloroplast genome based on 20 endonucleases; these *mat*K sequences provided greater resolution than did *rbc*L sequences (Johnson & Soltis, 1994). Herein we explore further the phylogenetic utility of *mat*K sequence variation through: (1) phylogenetic analyses of an expanded data set for Saxifragaceae s. s., a preliminary data set for *Gilia* (Polemoniaceae), and analysis of the polyploid origin of *Saxifraga osloensis* Knaben; (2) description of *mat*K sequence variation; and (3) discussion of the broad applicability of *mat*K sequencing primers in various plant groups.

Saxifragaceae s. s. continue to provide an ideal opportunity for assessing the utility of other chloroplast data sets because the family is well defined by molecular data, and both *rbc*L sequences and cpDNA restriction sites have been previously gathered for virtually all genera (e.g., Soltis et al., 1991; Soltis et al., 1993; Morgan & Soltis, 1993). We have expanded our *mat*K sequence matrix for Saxifragaceae s. s. from that reported by Johnson & Soltis (1994) to include an additional 324 bp and 14 additional species yielding 1078 bp of sequence data for 45 taxa. Additional species of *Saxifraga* and *Chrysosplenium* have been included because these genera possessed the longest branch lengths in our previous study (Johnson & Soltis, 1994). Additional members of the *Heuchera* and *Boykinia* groups of genera were also included to explore more fully the degree of resolution obtainable with *mat*K sequences in these well-supported groups. We present analyses of *mat*K data alone, as well as the results of combined analyses involving *mat*K and *rbc*L sequences and cpDNA restriction sites (Soltis et al., 1993) to provide a comprehensive view of relationships in Saxifragaceae s. s. as suggested by these three cpDNA data sets. We also discuss the fine-scale resolution that *mat*K can provide using an example of polyploid evolution in *Saxifraga*.

In addition to Saxifragaceae s. s., we also illustrate the phylogenetic utility of *mat*K sequences using analyses of a preliminary data set for *Gilia* (Polemoniaceae). Steele & Vilgalys (1994) presented an analysis using *mat*K sequences to resolve relationships among genera of Polemoniaceae, but they did not address intrageneric relationships within any of these genera. *Gilia* is a large, morphologically diverse genus of questionable monophyly and affinities. In many respects, the approximately 70 species currently recognized in *Gilia* (Day, 1993a) are united more by the lack of synapomorphic characters that circumscribe any of the other 12 to 14 temperate genera rather than by any consistent set of characters unique to themselves. We compare our phylogenetic trees for *Gilia* based on *mat*K sequences to those obtained in a recent analysis of sequences obtained from the nuclear ribosomal DNA internal transcribed spacer regions (rDNA-ITS) that included this genus (Porter, 1993).

We describe the nature of sequence variation in *mat*K, including: (1) comparison of nucleotide variability between *mat*K and *rbc*L, *ndh*F, and the ITS regions; (2) comparison of transition : transversion ratios and substitution rates by codon position between *mat*K and *rbc*L; (3) determination of the degree of random structuring of variation (Archie, 1989a); and (4) determination of the number and phylogenetic distribution of insertion-deletion events.

Lastly, in an effort to promote the use of *mat*K by other investigators, we also discuss the applicability of PCR and sequencing primers in various taxonomic groups.

Materials and Methods

Amplification and Sequencing

DNA was isolated from all taxa (Appendix 1) using a CTAB buffer method (Doyle & Doyle, 1987) as modified by Soltis et al. (1991). Ampli-

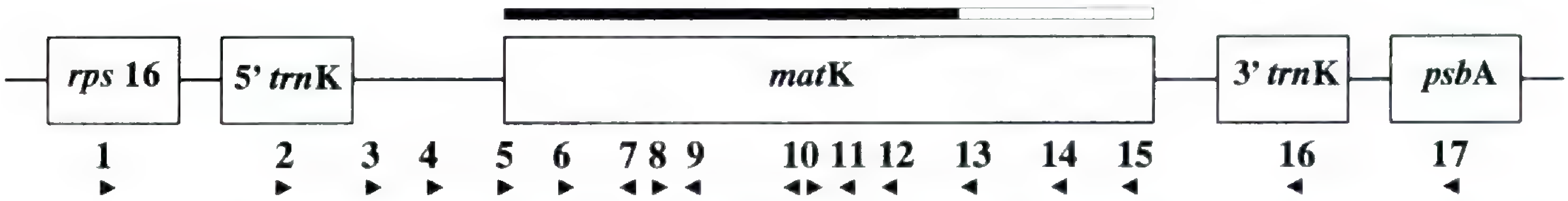


FIGURE 1. Relative location of amplification and sequencing primers used in this study for sequencing *mat*K and the *trn*K intron. Numbers refer to primers listed in Table 1. The shaded region above *mat*K indicates the region sequenced for species included in the Saxifragaceae s. s. and *Gilia* data matrices.

fication of *mat*K was accomplished via the polymerase chain reaction (PCR) to obtain sufficient quantities of DNA for sequencing. Several synthetic PCR primers located in the 5′*trn*K and 3′*trn*K exons, as well as in the *rps*16 and *psb*A genes that flank *trn*K (and thus *mat*K; Fig. 1, Table 1), have been designed. Two PCR primer pairs (*rps*16-4547F and *trn*K-2R; and *trn*K-3914F and *psb*A-R) provide DNA amplification products for initial sequencing of the entire *trn*K intron using *trn*K-3914F and *trn*K-2R, respectively, as sequencing primers (e.g., Johnson & Soltis, 1994). In this fashion, we sequenced the entire *trn*K intron in *Bensoniella oregona*, *Saxifraga integrifolia*, and *Sullivantia sullivantii* (a complete *mat*K sequence, excluding the flanking *trn*K intron regions, was also generated for *Sullivantia oregana*). For all other taxa, we used *trn*K-3914F and *trn*K-2R to produce double-stranded DNA from total genomic DNA. We subsequently used this double-stranded DNA as a template to produce single-stranded DNAs using primer *trn*K-3914F individually for forward strand synthesis, and primer *trn*K-2R for reverse strand synthesis. All PCR reactions used Replitherm polymerase from Epicentre Technologies following the manufacturer's suggested concentrations of all reagents and DNA. The PCR temperature profile we employed consisted of 30 cycles at 94°C for 1 minute 30 seconds, 48°C for 2 minutes, and 72°C for 3 minutes with an additional 15 minutes at 72°C following the final cycle. Secondary bands were occasionally observed in agarose test gels of our single-stranded DNA amplification products but only rarely in our double-stranded DNA products. These secondary bands never posed any problems during sequencing.

TABLE 1. Base composition of amplification and sequencing primers discussed in this study. Primers *rps*16-4547F and *trn*K-3914F (monocot) were designed by Jerry Learn. We later modified *trn*K-3914F (monocot) to produce *trn*K-3914F (dicot) and hence provide greater homology to dicots. Primers *trn*K-2R and *psb*A-R were designed by Kelly Steele. All other primers were designed in our lab and named based on their approximate position on the *trn*K map for *Sinapis* (Neuhaus & Link, 1987). Primer reference numbers correspond to those used in Figure 1.

Reference number in Figure 1	Name	5′ Sequence 3′
1	*rps*16-4547F	AGG TGC TCA ACC TAC AAG AAC C
2	*trn*K-3914F (dicot)	GGG GTT GCT AAC TCA ACG G
2	*trn*K-3914F (monocot)	ATC TGG GTT GCT AAC TCA ATG G
3	*trn*K-253F	TTG GGT CGA GTC AAT AAA T
4	*trn*K-582F	CTA ACC ATC TTG TTA TCC T
5	*trn*K-710F	GTA TCG CAC TAT GT[T/A] TCA TTT GA
6	*mat*K-934F	ATT TTG GTT ATG ACA ATA A
7	*mat*K-1168R	ATT GAA TGA ATT GAT CGT A
8	*mat*K-1176F	CAA TTC ATT CA[A/C] TAT TTC CTT
9	*mat*K-1235R	GG[A/G] GTG GGG TAT TAG TAT A
10	*mat*K-1412F	ATA TAA TTC TTA TGT ATG TG
10	*mat*K-1412R	CAC ATA [G/C]AT AAG AAT TAT AT
11	*mat*K-1470R	AAG ATG TTG AT[T/C] GTA AAT GA
12	*mat*K-1506R	TTC CAT AGA AAT ATA TTC G
13	*mat*K-1848R	TAT CGA ACT TCT TAA TAG C
14	*mat*K-2000R	ATT TCT GCA TAT GCG CAC AAA TC
15	*mat*K-2200R	TCT GTA TAA CCT CCA CAA AG
16	*trn*K-2R	AAC TAG TCG GAT GGA GTA G
17	*psb*A-R	CGC GTC TCT CTA AAA TTG CAG TCA T

Following PCR amplification, single-stranded DNAs were precipitated with 20% PEG/2.5M NaCl, washed in 70% and 95% EtOH, dried, and resuspended in TE (Morgan & Soltis, 1993). We subsequently employed dideoxy sequencing of the resuspended PCR products using the Sequenase 2.0 kit (U. S. Biochemical Corp.) and a set of sequencing primers (primers *mat*K-1168R or *mat*K-1235R, *mat*K-1470R, and *mat*K-1412F; or *mat*K-1168R, *mat*K-1506R, and *mat*K-1848R; Fig. 1; Table 1). These primers enable us to sequence routinely over two-thirds of *mat*K, beginning at the 5′ end (Fig. 1). Approximately 500 bp at the 3′ end were not sequenced because we have obtained sufficient resolution to address our present inquiries with the approximately 1080 bp obtained using the sequencing primers described above.

PLANT SAMPLES

Saxifragaceae s. s. In addition to extending our previously reported *mat*K sequences (Johnson & Soltis, 1994) by 324 bp, we sequenced 14 new species for a total of 45 sequences representing virtually all of Saxifragaceae s. s. (Appendix 1). Despite repeated attempts, we have been unable to obtain suitable material for DNA isolation from the monotypic genera *Hieronymusia*, *Saxifragella*, and *Zahlbrucknera* due to their geographically remote and restricted distributions. *Oresitrophe* is also missing from this analysis because we were not able to obtain material from this monotypic genus until after the lengthy phylogenetic analyses of Saxifragaceae s. s. were completed. *Tetracarpaea* and *Ribes* (Grossulariaceae) were included as outgroups because previous studies have shown these genera to be close relatives of Saxifragaceae s. s. (Morgan & Soltis, 1993; Soltis et al., 1993). Partial sequences of *Saxifraga osloensis*, *S. tridactylites*, and *S. adscendens* have also been obtained and are discussed below as an example of the insights *mat*K sequences can provide regarding polyploid origins; these sequences were not, however, included in our broad parsimony analyses of Saxifragaceae s. s.

Partial *mat*K sequences for Saxifragaceae s. s. vary from 1039 to 1063 bp in length and provide a matrix of 1078 characters after alignment. Ten insertion-deletion events (indels) of three, six, or nine nucleotides distributed among 18 species account for the length variation in these sequences (Appendix 2). We easily aligned these sequences visually and positioned indels so as to minimize base substitutions while maintaining the proper reading of codons. After alignment, we checked the reading frame and position of indels by translating the sequences to amino acids using either MacClade version 3.01 (Maddison & Maddison, 1992) or MEGA version 1.01 (Kumar et al., 1993). We scored missing bases associated with indels as ambiguous ("?"), rather than as a fifth character-state, and considered the phylogenetic distribution of each indel a posteriori by mapping its occurrence on trees derived from analysis of base substitutions alone.

These *mat*K sequences are the third chloroplast DNA data set constructed for the purpose of resolving relationships within Saxifragaceae s. s. A combined analysis of the other two data sets, cpDNA restriction sites and *rbc*L sequences, has recently been reported (Soltis et al., 1993). Here we compare and also combine our *mat*K sequences with these two other DNA data sets to obtain a comprehensive view of relationships suggested by cpDNA data in Saxifragaceae s. s. Because slight differences exist in the taxa sampled for the three molecular analyses (cpDNA restriction sites and *mat*K and *rbc*L sequences), we constructed three different combined matrices (Appendix 1) in an effort to obtain the most comprehensive combined analysis possible. Matrix-1 is the "purest" combined data set comprising 21 species for which all three character sets exist. Matrix-2 comprises all of the taxa from matrix-1 with the addition of *Astilbe* and *Chrysosplenium*, genera for which different species were analyzed in the various DNA studies. A complete data set for *Astilbe* was formed by combining the *rbc*L sequence and restriction site data from *A. taquetii* with the *mat*K sequence from *A. japonica* × *chinesensis*. Similarly, a complete data set for *Chrysosplenium* was formed by combining *mat*K and *rbc*L sequences from *C. iowense* with restriction site data from *C. americanum*. This approach seemed reasonable given that both *Astilbe* and *Chrysosplenium* are distinctive, well-defined genera in Saxifragaceae. We thus feel the likelihood of conflict among the character sets for these genera is not substantially greater than that existing among character sets for any of the other taxa for which a single species was used to generate all three data sets. Matrix-3 contains the broadest sampling of saxifragaceous genera, including all of the taxa in matrix-1 and an additional 22 taxa for which any two of the three character sets were available. For example, *Suksdorfia violacea* is included in matrix-3 because both cpDNA restriction site and *mat*K sequence data are available but not *rbc*L sequence data. Similarly, *Astilbe* and *Chrysosplenium* are represented in this matrix only by the character sets obtained from *A. taquetii* (cpDNA

restriction site and *rbc*L sequence data) and *C. iowense* (*mat*K and *rbc*L sequence data), rather than by the composite data set used in matrix-2. We filled the missing character set for each of the 11 taxa for which a character set is missing with "?" to indicate ambiguity for all missing characters.

Gilia. *Gilia* comprises approximately 70 species in six sections (sensu Day, 1993a, b). Although we are interested primarily in intersectional relationships involving sections *Saltugilia* and *Kelloggia*, *Gilia* has served as the catch-all among temperate Polemoniaceae (Grant, 1959; Wherry, 1940), and the *mat*K sequence results (see below) underscore the necessity of broad sampling among allied genera to gain the clearest picture of relationships in *Gilia*. Our preliminary data matrix for *Gilia* includes 31 species representing all six sections of *Gilia* (sensu Day, 1993a, b) and eight allied temperate genera (*Allophyllum*, *Collomia*, *Eriastrum*, *Ipomopsis*, *Langloisia*, *Navarretia*, *Phlox*, and *Polemonium*; Appendix 1). We selected the tropical genera *Bonplandia* and *Cantua* as outgroups because both traditional (e.g., Grant, 1959) and molecular data (Porter, 1993; Steele & Vilgalys, 1994) suggest these genera are close allies of the temperate Polemoniaceae.

The *mat*K sequences generated for *Gilia* vary from 1065 to 1080 bp in length and provide a matrix of 1083 characters after alignment. Four indels of three, six, or nine nucleotides in length distributed among eight taxa account for the length variation in these sequences (Appendix 2). As with the Saxifragaceae, we scored all missing bases as ambiguous ("?") and considered the phylogenetic distribution of indels after parsimony analysis of the base substitutions alone.

PHYLOGENETIC ANALYSES

PAUP (version 3.1.1, Swofford, 1991) installed on a Macintosh Centris computer was used to search for most parsimonious trees, perform bootstrap analyses (Felsenstein, 1985; see also Felsenstein & Kishino, 1993; Hillis & Bull, 1993) and decay analyses (Bremer, 1988; Donoghue et al., 1992), and to calculate the retention index (RI, Farris 1989a, b; see also Archie, 1989b, 1990) and consistency index (CI, Kluge & Farris, 1969) with autapomorphies retained. Parsimony analyses employed heuristic searches using TBR branch swapping, MULPARS, HOLD = 5 and SIMPLE addition. Five hundred replications of RANDOM addition with TBR branch swapping and MULPARS were also employed to search for multiple islands of most parsimonious trees (Maddison, 1991). Bootstrap analyses for each data set consisted of 100 replications using heuristic searches with TBR branch swapping, SIMPLE addition, and HOLD = 5. For our Saxifragaceae s. s. data set of *mat*K sequences, we additionally set a MAXTREE limit of 1000 trees per replicate after the computer ran out of memory on its 38th replication in an earlier analysis. Because few of the replications reached this limit and we used the bootstrap as only one estimate of support for monophyletic groups, we do not feel this restriction compromises the integrity of our results. Decay analyses were performed for all data sets using the following two approaches. For all data sets except the *mat*K sequence matrix for Saxifragaceae s. s. and the combined cpDNA data matrix-3, TBR branch swapping and 100 RANDOM addition heuristic searches that saved all trees up to five steps longer than the most parsimonious tree length were performed. Strict consensus trees were formed from these trees for each length greater than the most parsimonious tree length after filtering out trees of inappropriate length. This approach was inadequate, however, for the *mat*K sequence matrix and the combined cpDNA data matrix-3 for Saxifragaceae s. s. because of computer memory constraints and the large number of trees even a few steps greater than the most parsimonious tree length. For these data matrices, the decay analysis was conducted successively for each step greater than the most parsimonious tree length for up to five steps by saving only trees from heuristic searches that failed to satisfy a constraint topology imposed by the strict consensus of all trees found in the previous steps (Johnson & Soltis, 1994; Morgan et al., in press; Soltis & Kuzoff, in press).

VARIABILITY ANALYSES

Comparisons between genes. To determine the distribution of base substitutions and relative variability of regions within *mat*K, we used the IBM-PC program MEGA to make pairwise comparisons of nucleotide differences per site in complete *mat*K and *rbc*L sequences from three saxifragaceous taxa: *Bensoniella oregona*, *Saxifraga integrifolia*, and *Sullivantia oregana*. Pairwise comparisons of nucleotide differences and amino acid differences were also made among these three species and an additional 22 saxifragaceous taxa for which *rbc*L and over two-thirds of *mat*K have been sequenced. Separate parsimony analyses of these 25 *mat*K and *rbc*L DNA sequences were performed to estimate homoplasy as indicated by

CI and RI and to compare transition : transversion and codon position ratios as calculated by MacClade over a most parsimonious tree for each data set.

The increasing availability of DNA sequences from regions other than *rbc*L also enabled us to conduct two additional small-scale comparisons between *mat*K and other DNA sequences. In one such analysis, we made pairwise comparisons of nucleotide substitutions per site between *mat*K and *ndh*F for *Coriandrum* (Apiaceae), *Griselinia* (Cornaceae), and *Hedera* (Araliaceae) using *ndh*F sequences kindly provided by Robert Jansen. A similar comparison was made between *mat*K and ITS sequences (ITS-1 and ITS-2 combined) for *Gilia leptalea*, *G. scopulorum*, and *G. splendens*.

Variation in Saxifragaceae s. s. and Gilia matK matrices. We calculated the transition: transversion and codon position ratios as reconstructed over a most parsimonious tree for both the Saxifragaceae s. s. and *Gilia mat*K sequence matrices. The consistency indices for the Saxifragaceae s. s. and *Gilia mat*K data sets were also calculated and compared to expected CI values derived from the regression equation of Sanderson & Donoghue (1989) after first removing all autapomorphies from each data set with the aid of a spreadsheet program.

To assess how the observed variation in our Saxifragaceae s. s. and *Gilia mat*K matrices is structured, we employed the randomization test of Archie (1989a; see also Källersjö et al., 1992). The length of the most parsimonious trees from analyses of both data sets was compared to the distribution of most parsimonious trees derived from analyses of 100 data sets created by randomly permuting character states within characters via the SHUFFLE option in MacClade. Analyses of each randomized data set consisted of two heuristic searches employing first SIMPLE addition, and then CLOSEST addition, HOLD = 5, MULPARS, TBR branch swapping, and MAXTREES = 50. The shorter of the two results was used as the most parsimonious tree length. This approach provided sufficient speed for determination of non-random structure in our large data sets and was more likely (based on our initial tests) to obtain a shorter average tree length from the sample of 100 randomized data sets than an approach using thorough (i.e., no MAXTREE limit) searches employing only SIMPLE or only CLOSEST addition. The average length of the most parsimonious trees from these randomized data sets was also used to calculate the homoplasy excess ratio (HER; Archie, 1989b) for both Saxifragaceae s. s. and *Gilia*.

Results

Phylogenetic analyses

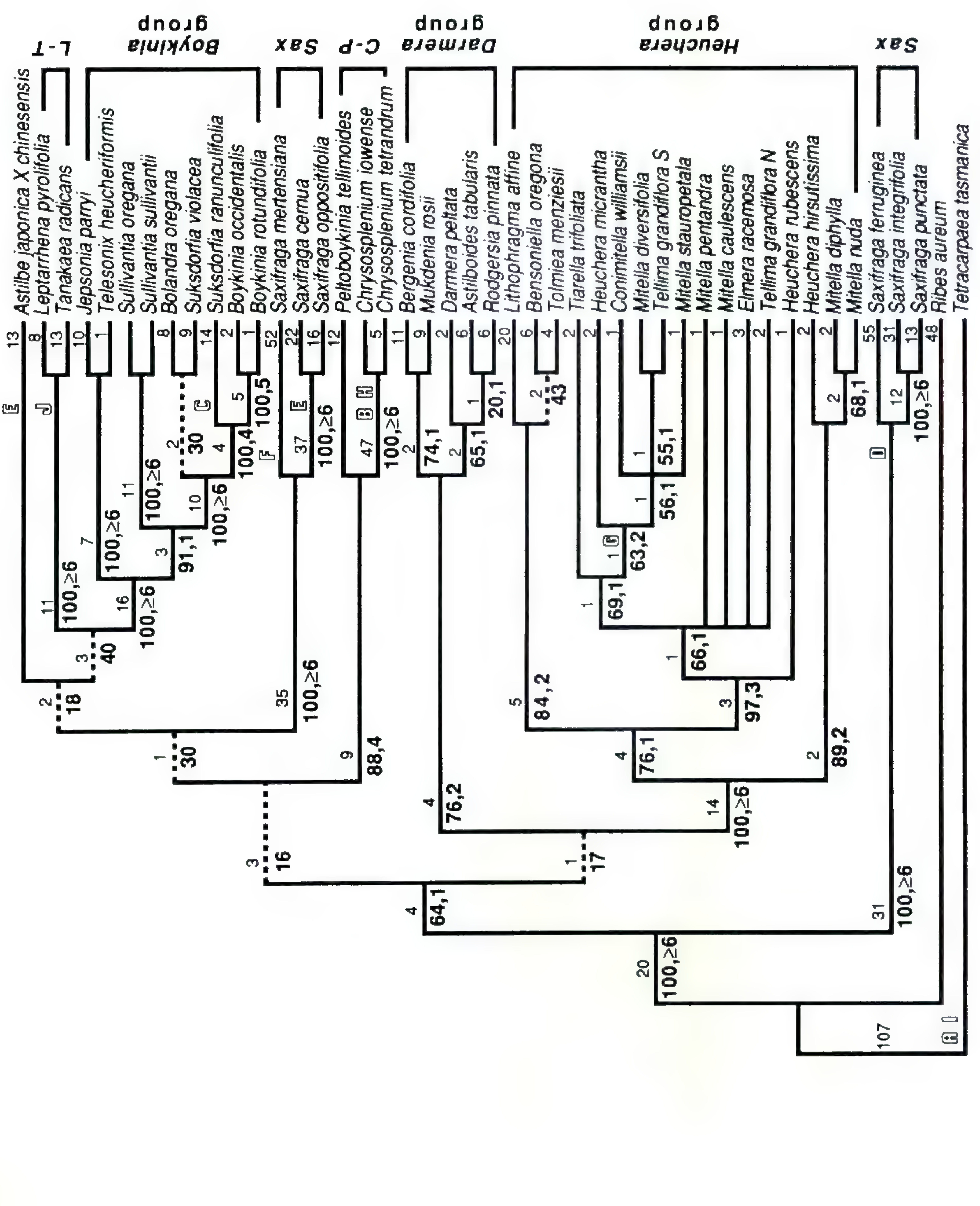
In analyses of all matrices, bootstrap and decay values generally agree in indicating support for monophyletic groups. Decay values ≥5 are rarely found on branches with bootstrap support of less than 95%, and bootstrap values ≥95% are rarely found on branches with decay values ≤3 (Figs. 2–6).

Saxifragaceae s. s. Parsimony analysis of *mat*K sequences for Saxifragaceae s. s. resulted in two islands with a total of 372 trees of 842 steps (Fig. 2). The consistency index excluding autapomorphies for these trees is 0.583, a higher value than that expected for 45 taxa using the regression equation of Sanderson & Donoghue (1989; Table 2). The large number of most parsimonious trees is primarily the result of homoplasy among the small number of substitutions supporting the basal branches. Increased homoplasy caused by the inclusion of a few highly divergent taxa also contributes to the large number of most parsimonious trees obtained. For example, although *Saxifraga mertensiana* is united with *S. cernua* and *S. oppositifolia* in all most parsimonious trees and the three taxa share 35 base substitutions (Fig. 2), removing *S. mertensiana* prior to parsimony analysis decreases the number of most parsimonious trees to 126.

Parsimony analyses of the combined cpDNA restriction site and *mat*K and *rbc*L sequence data sets for Saxifragaceae s. s. all yielded single islands of most parsimonious trees. Matrix-1 yielded two most parsimonious trees of 988 steps with a CI of 0.801 (autapomorphies retained; Fig. 3). Matrix-2 yielded a single most parsimonious tree of 1137 steps with a CI of 0.764 (autapomorphies retained;

→

FIGURE 2. One of 372 most parsimonious trees from analysis of *mat*K sequences for Saxifragaceae s. s. Base substitutions (ACCTRAN) are indicated above branches. Bootstrap and decay values are indicated below branches, respectively. Dashed lines represent branches that are not supported by all most parsimonious trees; in these instances the decay value is zero and is not indicated following the bootstrap value below the branch. Letters (A-J) denote the distribution of specific indels referenced in Appendix 2.


L-T
Boykinia group
Sax
C-P
Darmera group
Heuchera group
Sax
Astilbe japonica X chinesensis
Leptarrhena pyrolifolia
Tanakaea radicans
Jepsonia parryi
Telesonix heucheriformis
Sullivantia oregana
Sullivantia sullivantii
Bolandra oregana
Suksdorfia violacea
Suksdorfia ranunculifolia
Boykinia occidentalis
Boykinia rotundifolia
Saxifraga mertensiana
Saxifraga cernua
Saxifraga oppositifolia
Peltoboykinia tellimoides
Chrysosplenium iowense
Chrysosplenium tetrandrum
Bergenia cordifolia
Mukdenia rosii
Darmera peltata
Astilboides tabularis
Rodgersia pinnata
Lithophragma affine
Bensoniella oregona
Tolmiea menziesii
Tiarella trifoliata
Heuchera micrantha
Conimitella williamsii
Mitella diversifolia
Tellima grandiflora S
Mitella stauropetala
Mitella pentandra
Mitella caulescens
Elmera racemosa
Tellima grandiflora N
Heuchera rubescens
Heuchera hirsutissima
Mitella diphylla
Mitella nuda
Saxifraga ferruginea
Saxifraga integrifolia
Saxifraga punctata
Ribes aureum
Tetracarpaea tasmanica

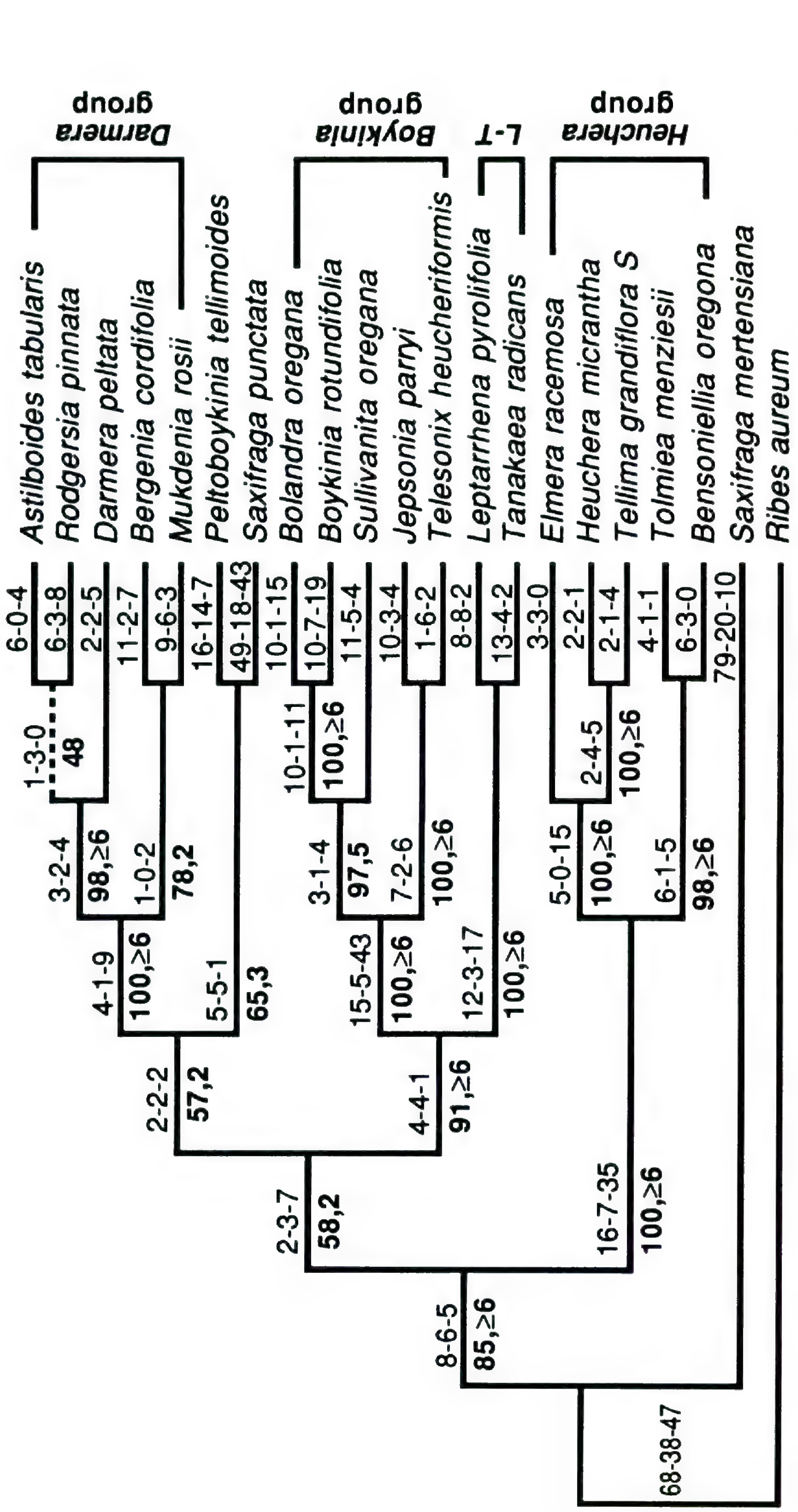


FIGURE 3. One of two most parsimonious trees for Saxifragaceae s. s. resulting from analysis of combined *matK* and *rbcL* sequences and cpDNA restriction site data for 21 taxa (combined matrix-1). Base substitutions (ACCTRAN) are indicated above branches and are partitioned by data set in the following order: *matK*-*rbcL*-cpDNA restriction sites. Bootstrap and decay values are indicated below branches, respectively. The dashed line represents the single branch that is not supported by both most parsimonious trees; the decay value is zero and is not indicated following the bootstrap value below the branch.

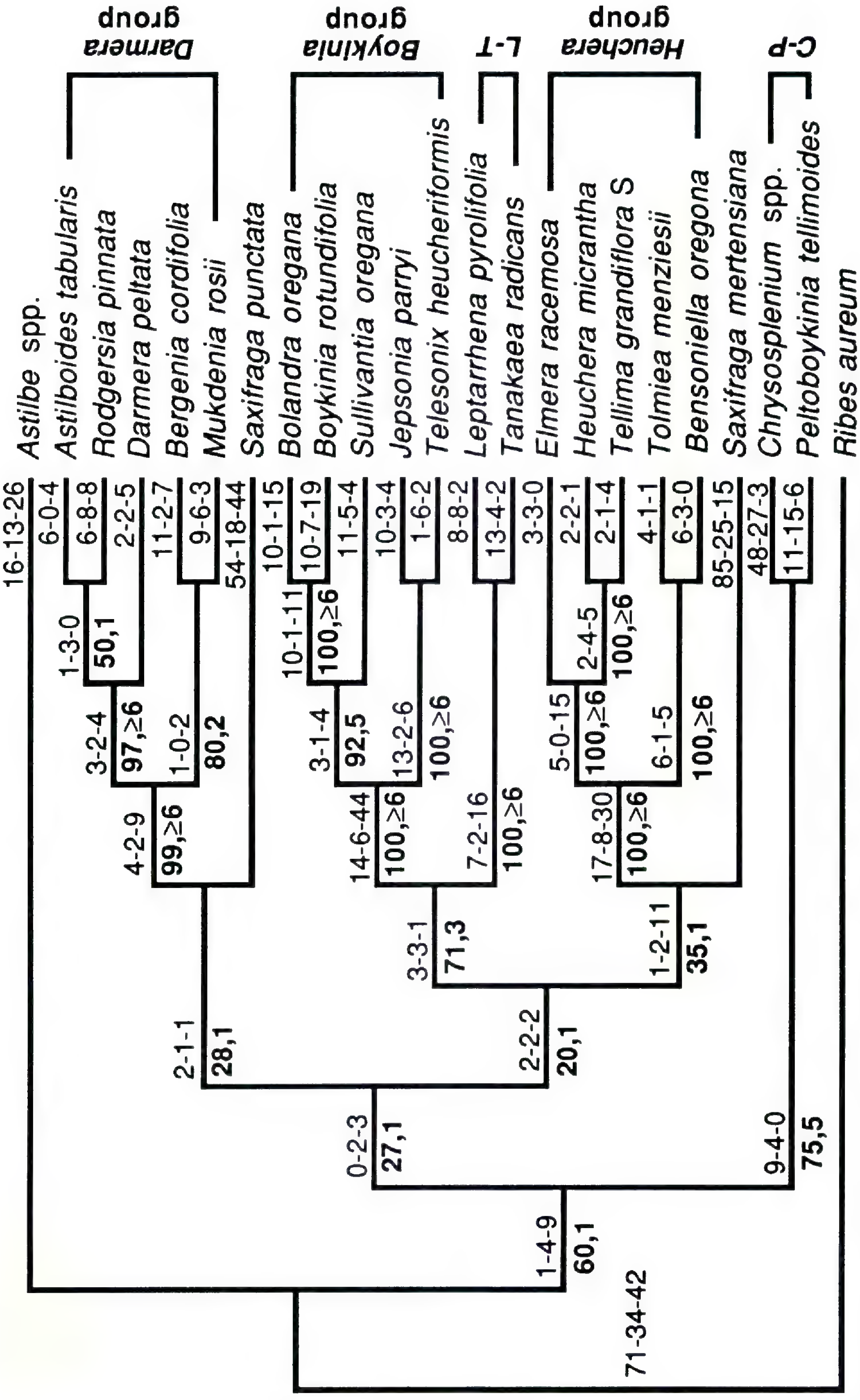


FIGURE 4. Single most parsimonious tree for Saxifragaceae s. s. resulting from analysis of combined *matK* and *rbcL* sequences and cpDNA restriction site data for 23 taxa (combined matrix-2). Base substitutions (ACCTRAN) are indicated above branches and are partitioned by data set in the following order: *matK-rbcL*-cpDNA restriction sites. Bootstrap and decay values are indicated below branches, respectively.

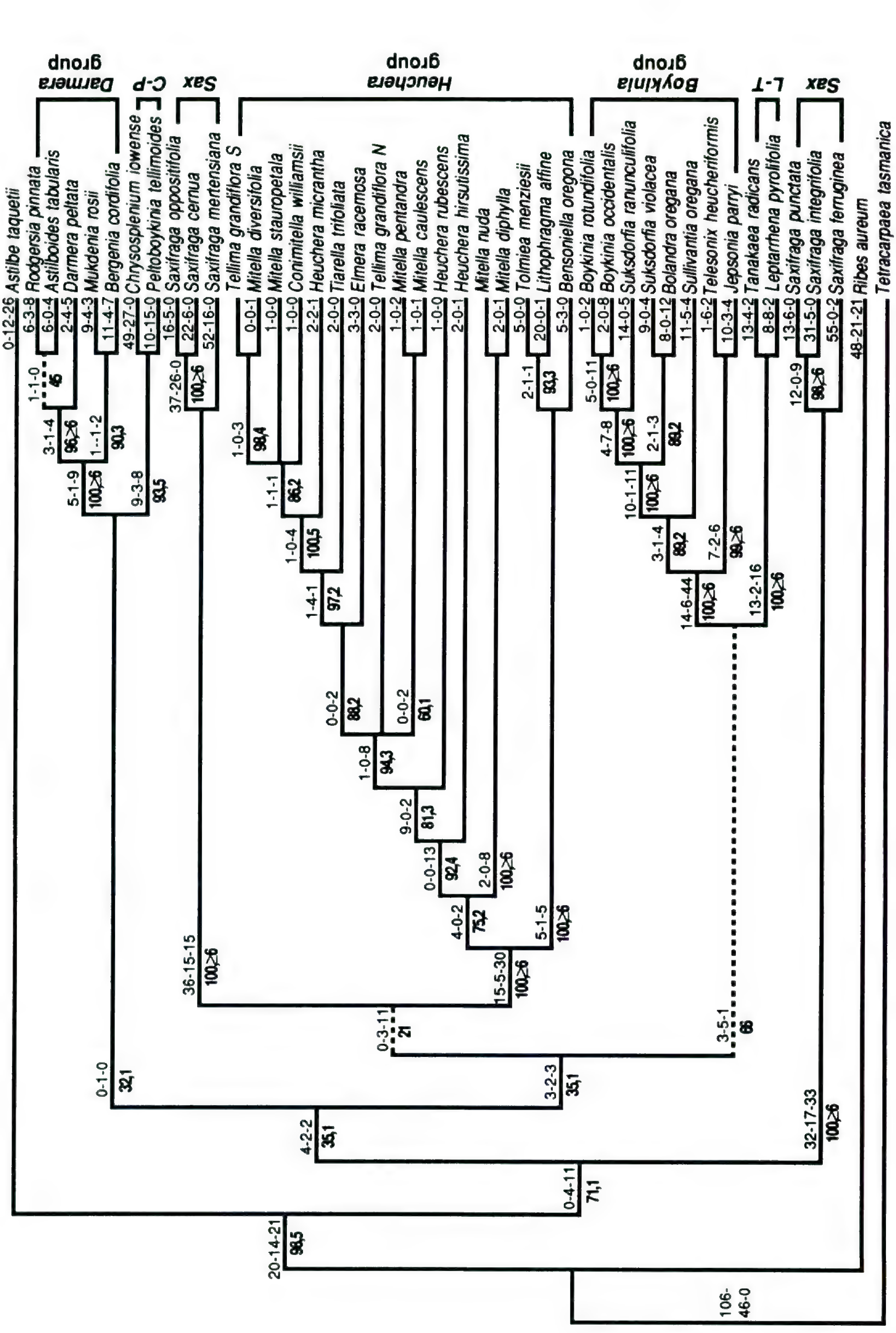

Astilbe taquetii
Rodgersia pinnata
Astilboides tabularis
Darmera peltata
Mukdenia rosii
Bergenia cordifolia
Darmera group
Chrysosplenium iowense
Peltoboykinia tellimoides
C-P
Saxifraga oppositifolia
Saxifraga cernua
Saxifraga mertensiana
Sax
Tellima grandiflora S
Mitella diversifolia
Mitella stauropetala
Conimitella williamsii
Heuchera micrantha
Tiarella trifoliata
Elmera racemosa
Tellima grandiflora N
Mitella pentandra
Mitella caulescens
Heuchera rubescens
Heuchera hirsutissima
Mitella nuda
Mitella diphylla
Tolmiea menziesii
Lithophragma affine
Bensoniella oregona
Heuchera group
Boykinia rotundifolia
Boykinia occidentalis
Suksdorfia ranunculifolia
Suksdorfia violacea
Bolandra oregana
Sullivantia oregana
Telesonix heucheriformis
Jepsonia parryi
Boykinia group
Tanakaea radicans
Leptarrhena pyrolifolia
L-T
Saxifraga punctata
Saxifraga integrifolia
Saxifraga ferruginea
Sax
Ribes aureum
Tetracarpaea tasmanica

Fig. 4). Matrix-3 yielded 24 most parsimonious trees of 1606 steps with a CI of 0.727 (autapomorphies retained; Fig. 5). Our analyses of these three matrices all demonstrated the presence of the same well-supported groups of genera (i.e., the *Boykinia* group, the *Darmera* group, the *Heuchera* group, and *Leptarrhena/Tanakaea*), groups also found in independent analyses of *mat*K sequences (Fig. 2) and *rbc*L sequences and cpDNA restriction sites (Soltis et al., 1993). In contrast, relationships among these well-supported groups of genera are poorly resolved, and there is considerable disagreement in relationships at basal branches among the shortest trees resulting from each of these three combined matrices (Figs. 3–5).

Gilia. Parsimony analysis of *mat*K sequences for *Gilia* yielded a single island of 36 most parsimonious trees each of 418 steps (Fig. 6). The consistency index excluding autapomorphies for these trees is 0.688, which is higher than the expected value for 31 taxa (Sanderson & Donoghue, 1989; Table 2).

VARIABILITY ANALYSES

Comparisons between genes. The average number of nucleotide differences per site in pairwise comparisons of entire (1518–1521 bp) *mat*K sequences for *Bensoniella oregona*, *Saxifraga integrifolia*, and *Sullivantia oregana* is 0.068. This value is 3.2 times greater than the average 0.021 nucleotide differences per site observed in pairwise comparisons of entire *rbc*L sequences for these same species. Comparisons of partial (747–845 bp) *mat*K sequences with *rbc*L (1392 bp) among gymnosperms (*Pinus Cunninghamia*, *Cunninghamia Widdringtonia*, *Widdringtonia Juniperus*, and *Juniperus–Microbiota*) similarly reveals an average 3.4 times greater level of nucleotide differences per site in *mat*K than in *rbc*L (P. Gadek & C. Quinn, pers. comm.).

Extending pairwise comparisons of nucleotide variability to 25 saxifragaceous taxa for which 1078 bp of *mat*K and all of *rbc*L has been sequenced gives an average 3.1-fold greater number of nucleotide differences per site in *mat*K as compared to *rbc*L. The lowest ratio (1.2 : 1) of nucleotide differences per site in this comparison occurred in two pairwise comparisons between members of the *Heuchera* group of genera (*Elmera–Tellima* and *Elmera–Heuchera*) that are only weakly differentiated by sequence data (Figs. 2 and 5). The highest ratio (6.2 : 1) of *mat*K to *rbc*L nucleotide differences per site also occurred within a single group of genera, the *Boykinia* group, between the well-differentiated genera *Bolandra* and *Jepsonia* (Figs. 2 and 5). When the percentage of base positions that are variable is compared across these 25 taxa, *mat*K has 3.2 times as many variable base positions and 2.7 times as many potentially informative characters as does *rbc*L (Table 3). The greater level of variation in *mat*K as compared to *rbc*L also extends to amino acids. Whereas 5% of amino acid positions are variable in *rbc*L, in *mat*K the figure is 59% (Table 3, Fig. 7). Although regions of high variability and regions that are more conserved are apparent in *mat*K sequences, variable sites appear to be fairly uniform in distribution throughout the 5′ portion of *mat*K that we have sequenced (Fig. 7). Character-state reconstructions over a most parsimonious tree for the *mat*K and *rbc*L data sets reveal that the variability in nucleotide substitutions is partitioned much more evenly in *mat*K with regard to transition : transversion and codon position ratios compared to *rbc*L (Table 3).

The average number of nucleotide differences per site in pairwise comparisons of *mat*K sequences (two-thirds complete) from *Coriandrum*, *Griselinia*, and *Hedera* are 1.3-fold greater than the average number observed in comparisons of entire *ndh*F sequences for these same species. The information content per gene is comparable between *ndh*F and *mat*K, however, given that *ndh*F is approximately 1.4 times as long as complete *mat*K sequences. In contrast, pairwise comparisons between *mat*K sequences from *Gilia leptalea*, *G. scopulorum*, and *G. splendens* and sequences from the combined nuclear ITS-1 and ITS-2 regions indicate an average 1.9-fold greater level of nucleotide differences per site in the ITS regions than in *mat*K. The information content of *mat*K is much greater than that of ITS, however, given that *mat*K

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FIGURE 5. One of 24 most parsimonious trees for Saxifragaceae s. s. resulting from analysis of combined *mat*K and *rbc*L sequences and cpDNA restriction site data for 43 taxa (combined matrix-3). Base substitutions (ACCTRAN) are indicated above branches and are partitioned by data set in the following order: *mat*K-*rbc*L-cpDNA restriction sites. Bootstrap and decay values are indicated below branches, respectively. Dashed lines represent branches that are not supported by all most parsimonious trees; in these instances the decay value is zero and is not indicated following the bootstrap value below the branch.

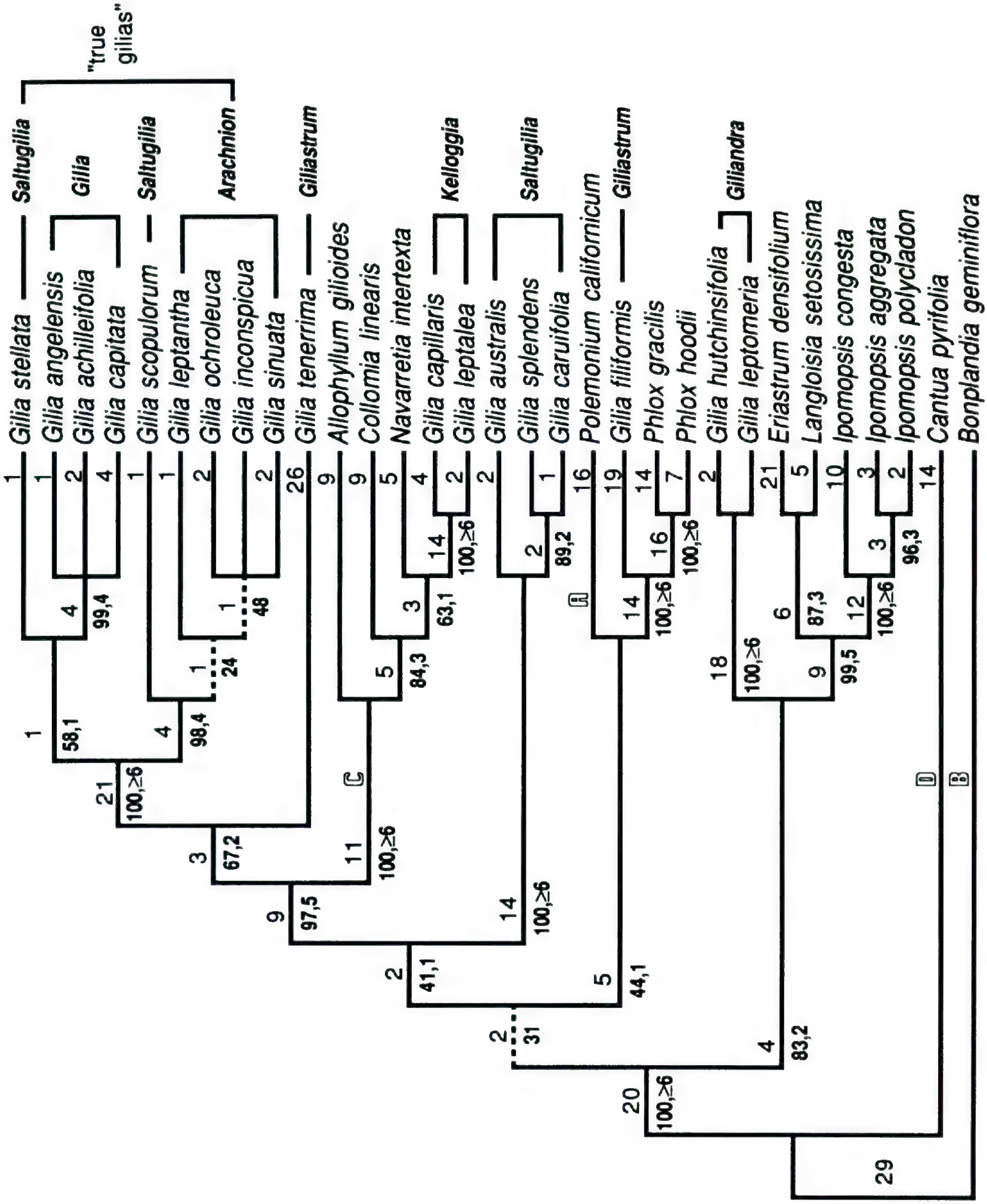
"true gilias"
Gilia stellata
Saltugilia
Gilia angelensis
Gilia achilleifolia
Gilia capitata
Gilia
Gilia scopulorum
Saltugilia
Gilia leptantha
Gilia ochroleuca
Gilia inconspicua
Gilia sinuata
Arachnion
Gilia tenerrima
Giliastrum
Allophyllum gilioides
Collomia linearis
Navarretia intertexta
Gilia capillaris
Gilia leptalea
Kelloggia
Gilia australis
Gilia splendens
Gilia caruifolia
Saltugilia
Polemonium californicum
Gilia filiformis
Giliastrum
Phlox gracilis
Phlox hoodii
Gilia hutchinsifolia
Gilia leptomeria
Giliandra
Eriastrum densifolium
Langloisia setosissima
Ipomopsis congesta
Ipomopsis aggregata
Ipomopsis polycladon
Cantua pyrifolia
Bonplandia geminiflora

TABLE 2. Summary of descriptive measures and indices of variation in our *mat*K sequence matrices for Saxifragaceae s.s. (45 taxa) and *Gilia* (31 taxa).

Measure	Saxifragaceae s.s.	*Gilia*
Number of characters	1078	1083
Percent of characters variable	44	26
Percent of characters potentially informative	25	16
Consistency index (CI)	0.735	0.785
CI excluding autapomorphies	0.583	0.688
Expected CI excluding autapomorphies (Sanderson & Donoghue, 1989)	0.341	0.423
Retention index (RI)	0.792	0.871
Homoplasy excess ratio (HER)	0.727	0.827
Transition : transversion ratio	1 : 1.09	1 : 1.05
Codon position ratio	1.17 : 1 : 1.58	1.44 : 1 : 1.87

is approximately 3.1 times longer than the ITS regions combined.

Variation in the noncoding trnK intron. To date, phylogenetic analyses using the *trn*K region have concentrated on *mat*K; however, the *trn*K intron regions flanking *mat*K (Fig. 1) may also have phylogenetic potential. Although we have not used the intron regions between the 5′ *trn*K exon and *mat*K (5′ intron region) and *mat*K and the 3′ *trn*K exon (3′ intron region) for phylogenetic analyses, we have sequenced the major portion of these regions for *Bensoniella oregona*, *Saxifraga integrifolia*, and *Sullivantia sullivantii*. The 5′ intron region is approximately 720 bp with an average 0.059 nucleotide differences per site in pairwise comparisons of these species. The 3′ region is approximately 200 bp with an average 0.116 nucleotide differences per site. For proper alignment of these three sequences, each region required the insertion of five gaps of one to nine bp. The observed variability in these regions flanking *mat*K is slightly less than *mat*K itself for the 5′ flanking region but considerably higher for the 3′ flanking region. Both regions may provide additional, useful phylogenetic information.

Variation in Saxifragaceae s. s. and Gilia matK matrices. As reconstructed over one of their most parsimonious trees (Figs. 2 and 6, respectively), the number of transitions was essentially equivalent to the number of transversions observed in both the *mat*K data set for Saxifragaceae s. s. and *Gilia* (Table 2). As expected, substitutions at the second codon position were less frequent than those at the first or third positions. However, the numbers of substitutions at the first and second codon positions were only slightly lower than the number observed at the third position in both the Saxifragaceae s. s. and the *Gilia mat*K data sets (Tables 2 and 3). In contrast, in *rbc*L the number of third position substitutions is much higher than the numbers of substitutions at the first and second codon positions, as is typical of protein-coding regions with strong functional constraints (Table 3; Donoghue et al., 1992; Smith et al., 1993; see also Steele & Vilgalys, 1994).

The randomization test of Archie (1989a) indicates that variation in the Saxifragaceae s. s. and *Gilia mat*K data sets is substantially non-randomly structured. The most parsimonious tree length (Fig. 2; 842 steps) obtained from analysis of the *mat*K sequences for Saxifragaceae s. s. is far removed from the range (1188 to 1213 steps) and mean (1200 steps) of the most parsimonious tree lengths derived from 100 random permutations of these data. Likewise, the most parsimonious tree length (418 steps) obtained from analysis of the *mat*K sequences for *Gilia* is far removed from the range (838 to 859 steps) and mean (847 steps) of the most parsimonious tree lengths derived from 100 random permutations of the *Gilia* data matrix. Because HER (Archie, 1989b) measures congruence among characters and departure from randomness (i.e., HER equals one when data are com-

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FIGURE 6. One of 36 most parsimonious trees from analysis of *mat*K sequences for *Gilia* and related genera. Base substitutions (ACCTRAN) are indicated above branches. Bootstrap and decay values are indicated below branches, respectively. Dashed lines represent branches that are not supported by all most parsimonious trees; in these instances the decay value is zero and thus is not indicated following the bootstrap value below the branch. Letters (A D) denote the distribution of specific indels referenced in Appendix 2.

TABLE 3. Comparison of sequence variation between *mat*K and *rbc*L for the same suite of 25 saxifragaceous taxa. Taxa included in this comparison are indicated in Appendix 1.

Comparison	*mat*K	*rbc*L
Number of characters	1078	1398
Percent of characters variable	38	12
Percent of characters potentially informative	16	7
Percent of amino acid positions variable	59	5
Number of most parsimonious trees	3	24
Consistency index (CI)	0.790	0.712
CI excluding autapomorphies	0.635	0.580
Retention index (RI)	0.736	0.699
Transition : transversion ratio	1 : 1.06	1.41 : 1
Codon position ratio	1.22 : 1 : 1.57	1.26 : 1 : 6.17
RI by codon position	0.76; 0.71; 0.73	0.57; 0.63; 0.75

pletely congruent and approaches zero as data approach randomness in the distribution of character states), we use HER as an indicator of the degree of hierarchical structure present in our data matrices. Values of HER are 0.727 and 0.827 for our Saxifragaceae s. s. and *Gilia matK* data sets, respectively. These values indicate that homoplasy and departure from hierarchical structure in these *matK* data are approximately 27.3% (in Saxifragaceae s. s.) and 17.3% (in *Gilia*) of that present in randomly structured data sets possessing the same character-state distributions as our original matrices.

DISCUSSION

PHYLOGENETIC ANALYSES

Saxifragaceae s. s. Several recent studies have presented phylogenetic trees based on *rbcL* sequences and cpDNA restriction sites for members of Saxifragaceae s. s. In addition, the implication of these cpDNA-based phylogenetic trees regarding trends in chemical, morphological, and cytological evolution within the family have been discussed. Herein we focus on comparisons between previous phylogenetic trees based on *rbcL* sequences and cpDNA restriction site variation (Morgan & Soltis, 1993; Soltis et al., 1993) and relationships suggested by *matK* sequences when analyzed both independently and combined with the other two data sets.

Strong support for the monophyly of Saxifragaceae s. s. has been demonstrated by an extensive analysis of *rbcL* sequences representing a diverse array of dicots (Morgan & Soltis, 1993), as well as with *rpl2* intron data (Downie et al., 1991). Parsimony analysis of 1078 bp of *matK* sequence for 45 taxa also reveals a well-supported Saxifragaceae s. s. (Fig. 2). The members of Saxifragaceae s. s. share seven unique base substitutions, are united in all trees found during 100 bootstrap replications, and form a clade in all trees up to at least six steps longer than the most parsimonious trees (Fig. 2), relative to the two outgroup taxa chosen.

Within Saxifragaceae s. s., several lineages and groups of genera (i.e., the *Boykinia*, *Heuchera*, and *Leptarrhena/Tanakaea* groups, and two lineages of *Saxifraga*) are well supported by *matK* sequences, whereas other groups of genera (i.e., the *Darmera* and *Chrysosplenium/Peltoboykinia* groups) are less well supported but consistently united in all most parsimonious trees (Fig. 2). Although we have increased both the number of nucleotides and the number of taxa in the *matK* matrix by almost 50% over an earlier analysis (Johnson & Soltis, 1994), relationships among all of the major lineages noted above remain unresolved. Low support for those branches uniting the major groups of genera was also evident in independent analyses of *rbcL* sequences and cpDNA restriction sites (Soltis et al., 1993) and in the combined analyses of all three cpDNA data sets (Figs. 3–5). Whereas the inability of these three cpDNA data sets to elucidate relationships among the major groups of genera in Saxifragaceae s. s. may seem unsatisfactory from both a cladistic and a taxonomic standpoint, it nonetheless is significant in that it supports the occurrence of a rapid radiation early in the evolutionary history of Saxifragaceae s. s., an event also suggested by other lines of evidence (e.g., host preferences of *Puccinia* rusts; Savile, 1975).

In contrast to the lack of support among the major groups of genera, relationships within some of these groups are well supported by *matK* sequences. Phylogenetic relationships among members of the *Boykinia* group are, for example, most-

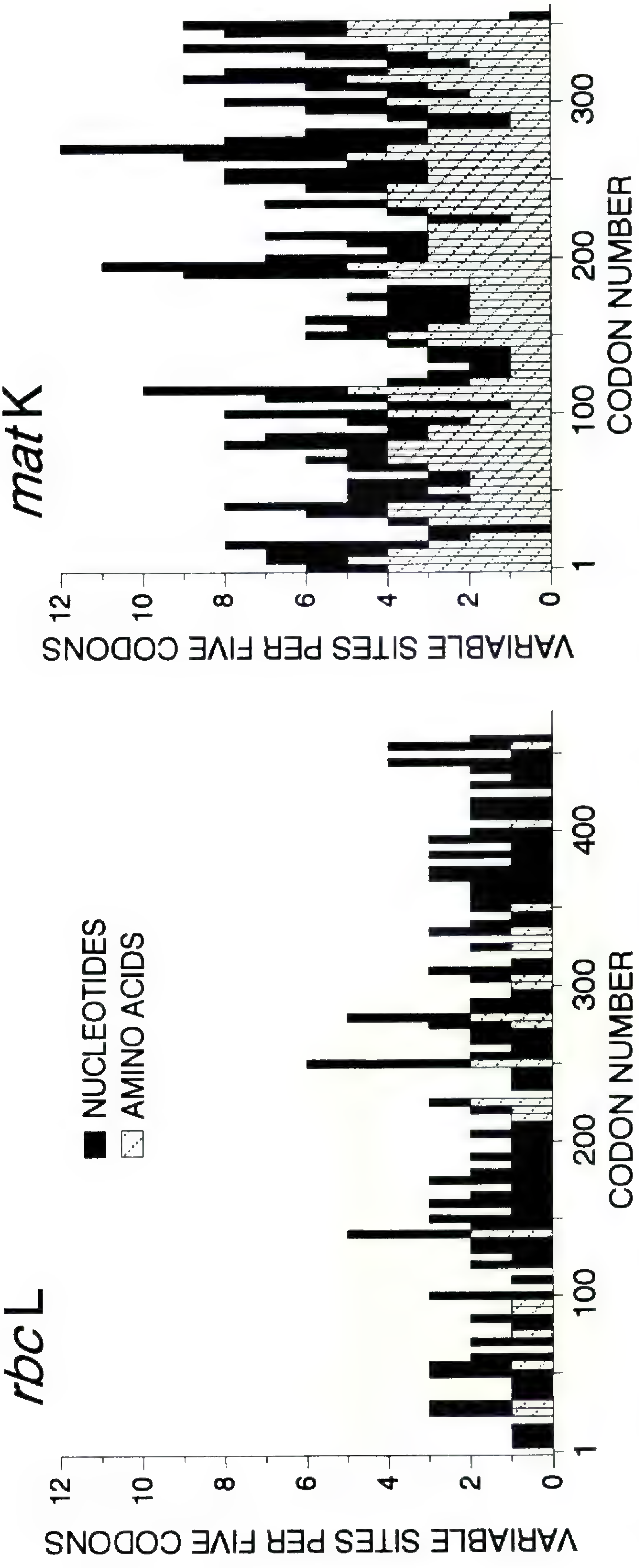

FIGURE 7. Variable nucleotide and amino acid sites per five codons in *rbc*L and the 5′ two-thirds of *mat*K for the same set of 25 saxifragaceous taxa (see also Table 3).

ly well defined and strongly supported by bootstrap values of 100 and decay values of four or higher (Fig. 2). Significantly, the degree of support for generic- and species-level relationships within the *Boykinia* group based on *mat*K sequences is comparable to that obtained via analysis of cpDNA restriction sites (Soltis et al., 1993); these same strongly supported relationships are also seen in the analysis of the three combined cpDNA data sets (Fig. 5). Trees based on *mat*K sequences (Fig. 2) agree with those obtained from analysis of cpDNA restriction sites: in recognizing a strong relationship between *Telesonix* and the enigmatic *Jepsonia*; in suggesting that *Sullivantia* is sister to *Bolandra*, *Boykinia*, and *Suksdorfia*; and in indicating the polyphyly of *Suksdorfia*.

Phylogenetic analysis of *mat*K sequences not only indicates that *Saxifraga* is polyphyletic, comprising two well-supported lineages, but also reveals considerable differentiation among species within each of these two lineages (Fig. 2). *Saxifraga integrifolia*, *S. ferruginea*, *S. mertensiana*, and *S. punctata* (= *S. nelsoniana*) have traditionally been placed in section *Micranthes* (Engler & Irmscher, 1916–1919). Gornall (1987), however, considered *S. mertensiana* so distinctive morphologically that he placed it in its own section, *Heterisia*. Significantly, *S. mertensiana* is well removed from these three species of section *Micranthes* in all most parsimonious trees derived from analysis of *mat*K sequences (Fig. 2) and differs from these species by a minimum of 135 nucleotide differences and 2 indels. The distant relationship of *S. mertensiana* to members of section *Micranthes* (Fig. 2) is also suggested by independent analyses of *rbc*L sequences and cpDNA restriction sites (Soltis et al., 1993), as well as analyses of the combined cpDNA data sets (Figs. 3–5). Additionally, the sister relationship suggested by analysis of *mat*K between *S. mertensiana* and *S. cernua/S. oppositifolia* (of sections *Saxifraga* and *Porphyrion*, respectively) is also suggested by *rbc*L sequences (Soltis et al., 1993). Given the large number (35) of base substitutions uniting *S. mertensiana* with *S. cernua/S. oppositifolia* and an even larger number (52) of autapomorphies in *S. mertensiana*, we cannot discount the possibility that this relationship is an artifact produced by attraction of long branches during parsimony analysis (Felsenstein, 1978). Nonetheless, the level of differentiation in *mat*K observed among the small sample of *Saxifraga* species included herein (Fig. 2; range of 44 to 162 base substitutions in pairwise comparisons) suggests that additional comparative *mat*K sequencing within *Saxifraga* should be extremely useful at defining lineages and relationships among many of the 300 species that are presently recognized in this morphologically diverse genus.

A fourth well-supported, albeit small, lineage in Saxifragaceae s. s. comprises the sister genera *Leptarrhena* and *Tanakaea* (Fig. 2). Previous analyses of *mat*K sequences (Johnson & Soltis, 1994) and *rbc*L sequences (Soltis et al., 1993), as well as the analysis of the three combined cpDNA data sets (Figs. 3–5), not only support this relationship, but also suggest that these two genera are sister to the *Boykinia* group. This relationship to the *Boykinia* group is generally not strongly supported, however, and is not revealed in all most parsimonious trees from analysis of our expanded *mat*K data set (Fig. 2), analysis of combined cpDNA data matrix-3 (Fig. 5), or analysis of cpDNA restriction site data (Soltis et al., 1993). Matrix-1 of the combined cpDNA data sets (Fig. 3) provides the strongest support for a sister relationship between *Leptarrhena/Tanakaea* and the *Boykinia* groups (91% bootstrap and decay value ≥6). However, the small number of taxa in this matrix does not include what may be critical taxa for defining the true affinities of *Leptarrhena/Tanakaea*. For example, matrix-2 of the combined cpDNA data sets includes just two additional genera, *Astilbe* and *Chrysosplenium*, yet support for *Leptarrhena/Tanakaea* as sister to the *Boykinia* group declines to 71% bootstrap and a decay value of 3 in analyses of this data set (Fig. 4).

The *Heuchera* group of genera is strongly supported by *mat*K sequences, and several lineages are differentiated within this group. As was observed in an earlier analysis of cpDNA restriction site data (Soltis et al., 1991), *Heuchera* and *Mitella* appear polyphyletic with species located in several independent lineages. Both cpDNA restriction sites and *mat*K sequences also provide strong support for a clade comprising *Bensoniella*, *Lithophragma*, and *Tolmiea*. *mat*K and cpDNA restriction site data sets do not concur, however, in the affinities of *Bensoniella*, *Lithophragma*, and *Tolmiea* within the *Heuchera* group. Analysis of cpDNA restriction sites suggests that *Bensoniella*, *Lithophragma*, and *Tolmiea* are the sister to all other members of the *Heuchera* group. In contrast, analysis of *mat*K sequences suggests that *Bensoniella*, *Lithophragma*, and *Tolmiea* are the sister to *Conimitella*, *Elmera*, *Tellima*, *Tiarella*, and most species of *Heuchera* and *Mitella*; all of these taxa then appear as the sister to *Heuchera hirsutissima*, *Mitella diphylla*, and *M. nuda* (Fig. 2). The latter three species are well differentiated from other species of *Heuchera* and *Mitella* in the

cpDNA restriction site study (Soltis et al., 1991). The unusual position of these three species in the *Heuchera* group suggested by *mat*K sequences requires further investigation.

Analysis of *mat*K sequences provides support for *Chrysosplenium* and *Peltoboykinia* as sister genera (Fig. 2), a relationship that is also suggested by *rbc*L sequences but not by all shortest trees based on cpDNA restriction sites (Soltis et al., 1993). Not only is the degree of bootstrap support for this relationship high (88%), but these two genera are also united in all trees up to 4 steps longer than the most parsimonious tree and receive similar support in analyses of the combined cpDNA data sets (Figs. 4, 5). The sister relationship between these two genera is significant because both genera are morphologically distinctive and their affinities are problematic. *Chrysosplenium* is particularly well defined morphologically and contains a large number of species. It is thus noteworthy that the branch uniting the two *Chrysosplenium* species is among the longest observed on the *mat*K phylogenetic tree (Fig. 2), with 47 substitutions and two unique indels uniting these species. Comparison of a few partial sequences from other species of *Chrysosplenium* (D. Soltis, unpublished) suggests that *mat*K sequences will be useful for elucidating relationships within this genus.

The *Darmera* group of genera is also recognized based on analysis of *mat*K sequences, although this group is not as strongly supported (76% bootstrap, decay value of 2; Fig. 2) as are some of the other major groups of genera in Saxifragaceae s. s. The *Darmera* group is, however, well supported by cpDNA restriction sites (99% bootstrap, ≥6 steps of decay; Soltis et al., 1993) and also appears in our analyses of the three combined cpDNA data sets (Figs. 3–5). In contrast, *rbc*L sequences fail to recognize the *Darmera* group, although this appears to be the result of the small number of base substitutions in *rbc*L at this level of analysis rather than indicative of a strongly supported opposing view of relationships (Soltis et al., 1993). As within other major groups of genera in Saxifragaceae, *mat*K and cpDNA restriction sites again provide comparable pictures of relationships within the *Darmera* group. Both cpDNA restriction sites and *mat*K sequences indicate that *Bergenia* and *Mukdenia* are sister taxa and that *Astilboides*, *Darmera*, and *Rodgersia* are closest relatives.

Gilia. In a recent analysis of 661 bp of *mat*K sequence for 20 polemoniaceous taxa, *Gilia* appeared monophyletic (Steele & Vilgalys, 1994); however, Steele and Vilgalys included only four species of *Gilia* from two sections, *Arachnion* and *Gilia*. Our broader analysis of *mat*K sequences from species representing all six sections of *Gilia* reveals that species of *Gilia* are scattered throughout the temperate radiation of Polemoniaceae (Fig. 6). Species of *Gilia* appear in several well-separated lineages that also include members of virtually all of the other temperate genera included in this analysis (i.e., *Allophyllum*, *Collomia*, *Eriastrum*, *Ipomopsis*, *Langloisia*, *Navarretia*, *Phlox*, and *Polemonium*). Thus, our analysis clearly indicates that *Gilia* is polyphyletic. Although few of the species of *Gilia* included in the *mat*K analysis were identical to those sampled in a recent ITS sequence analysis of Polemoniaceae (Fig. 8; Porter, 1993), the sectional coverage of *Gilia* in the two studies is comparable. Both *mat*K and ITS phylogenetic trees agree in suggesting similar relationships among sections of *Gilia* and allied genera (compare Figs. 6 and 8). Furthermore, relationships that are only weakly supported by *mat*K sequence data, such as the branching patterns among basal nodes (Fig. 6), are also only weakly supported by ITS sequences (Fig. 8).

Analyses of *mat*K sequences strongly support a clade of "true gilias" comprising sections *Gilia*, *Arachnion*, and the species *G. scopulorum* and *G. stellata* of section *Saltugilia* (Fig. 6). A close relationship among sections *Arachnion*, *Gilia*, and *Saltugilia* has been previously suggested (Grant, 1954, 1959; Grant & Grant, 1956a, b). Furthermore, although both *G. scopulorum* and *G. stellata* have been allied with the *G. splendens* group of section *Saltugilia* (Grant & Grant, 1954, 1956b; Grant, 1959; Day, 1993b), the distinctness of the former species with regard to the *G. splendens* group has also been recognized (Grant & Grant, 1954, 1956b). ITS sequences also define a clade of true gilias similar in composition to that defined by *mat*K sequences. However, in the ITS analysis *G. stellata* and *G. scopulorum* are united in a single clade as sister to both sections *Gilia* and *Arachnion* (Fig. 8), rather than placed apart as suggested by *mat*K sequences (Fig. 6).

Gilia tenerrima (section *Giliastrum*) appears as a weakly supported sister to the true gilias in analyses of both *mat*K (Fig. 6) and ITS (Fig. 8) sequences. The morphology of *G. tenerrima* is unique among gilias, and its affinities have been sought among species currently recognized in section *Kelloggia* (e.g., Mason & Grant, 1948; Grant, 1959) or section *Giliastrum* (e.g., Grant & Grant, 1954; Day, 1993a). The polyphyly of section *Giliastrum* is indicated by *mat*K sequences based on the inclusion of only one other species of section *Gi-*

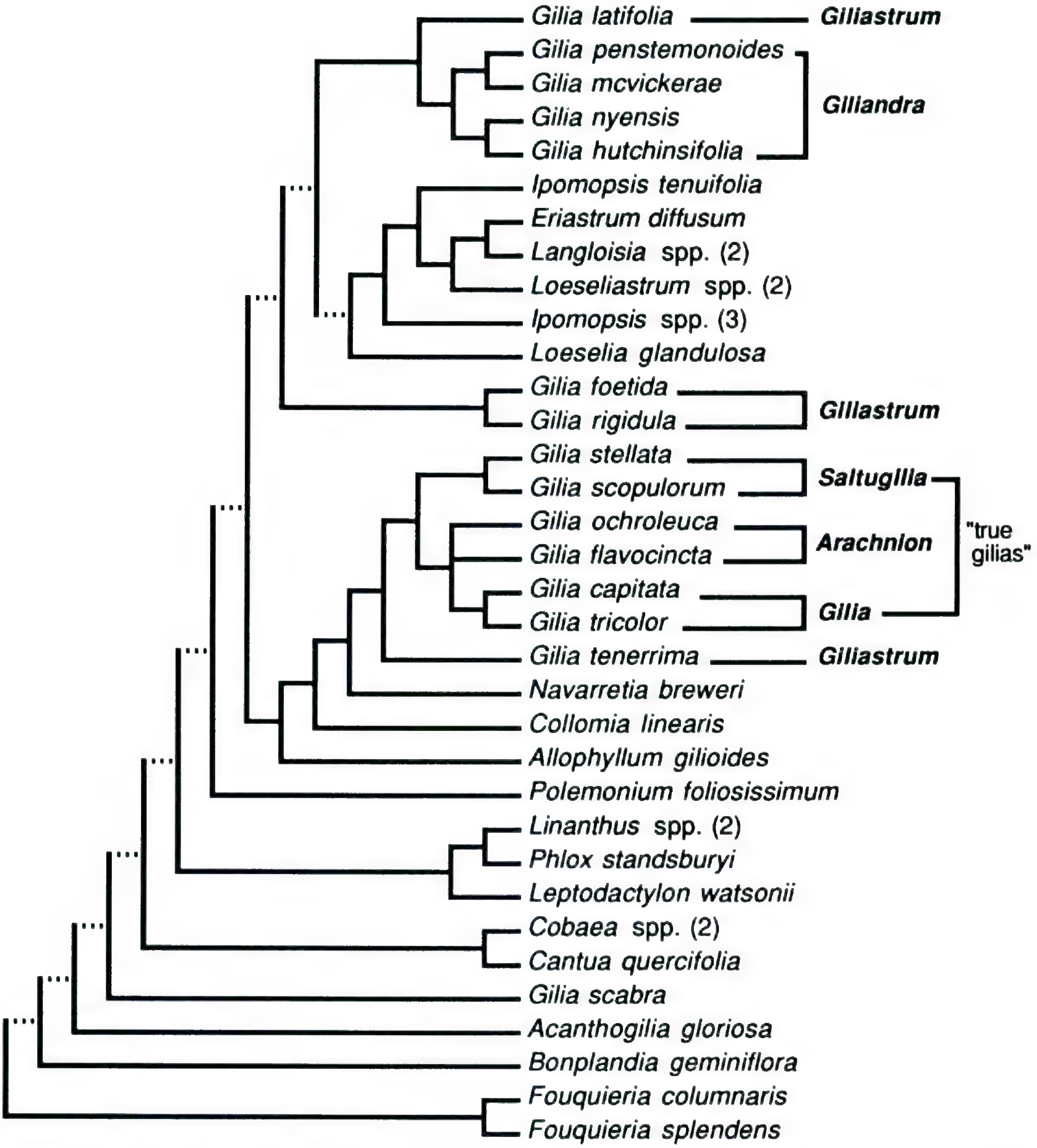

FIGURE 8. Strict consensus of two most parsimonious trees for Polemoniaceae resulting from parsimony analysis of nuclear rDNA ITS sequences (length = 1074; CI = 0.47). Poorly supported branches, as indicated by bootstrap, jackknife, decay, and skewness (Hillis & Huelsenbeck, 1992) analyses, are represented by broken lines. A few terminal branches have been collapsed to emphasize relationships discussed herein; in these cases, the value in parentheses to the right of the generic name represents the number of species that appear along the branch. Data analysis and tree courtesy of Porter (1993).

liastrum, *G. filiformis*, in this analysis. *Gilia filiformis* is strongly allied with a lineage including *Phlox* in the *mat*K analysis (Fig. 6) and is well separated from *G. tenerrima* by 65 bases in a pairwise comparison of nucleotide differences. The polyphyly of section *Giliastrum* is also strongly suggested by ITS sequences (Fig. 8).

A well-supported sister lineage to *G. tenerrima* and the clade of true gilias is a strongly supported group comprising *Allophyllum*, *Collomia*, *Navarretia*, and species of *Gilia* section *Kelloggia*. These taxa share a 6-bp deletion and 10 unambiguous base substitutions on the *mat*K phylogenetic tree (Fig. 6). *Gilia capillaris* and *G. leptalea* (section

Kelloggia; Mason & Grant, 1948; Day, 1993a) form a well-defined lineage within this group and are differentiated from their sister genera by eight unambiguous base substitutions. These two species have, until recently (Day, 1993a), been recognized as a distinct species group in section *Saltugilia* (Grant & Grant, 1954; Grant, 1959). Analyses of *mat*K sequences thus support the recognition of section *Kelloggia*, although the close relationship of this section to *Allophyllum*, *Collomia*, and *Navarretia* has not been considered in recent classifications. It is noteworthy, however, that Gray (1873) and Brand (1907) placed *G. leptalea* and *G. sinistra*, respectively, in *Collomia* (*G. sinistra* is also in section *Kelloggia* but was not included in this analysis of *mat*K sequences). Species from section *Kelloggia* were not included in Porter's (1993) ITS analysis, and ITS sequences place *Navarretia*, *Collomia*, and *Allophyllum* as progressively basal sisters to *G. tenerrima* and the true gilias rather than united as a single lineage as suggested by *mat*K sequences (Fig. 6). This apparent discrepancy between the ITS and *mat*K trees may simply be a result of inappropriate rooting of the ITS sequence clade that includes *Allophyllum* and the true gilias due to the inclusion of very divergent sequences in the ITS matrix (M. Porter, pers. comm.). For example, the *Allophyllum*-true gilia clade in the ITS tree (Fig. 8) could be rerooted at the midpoint of the branch between *G. tenerrima* and *Navarretia* to provide essentially the same view of relationships depicted by the *mat*K sequence tree (Fig. 6).

The core members of section *Saltugilia*, *G. splendens*, *G. australis*, and *G. caruifolia*, were also not included in Porter's (1993) ITS sequence analysis but are strongly supported by *mat*K data as a unique lineage well separated from all other lineages of *Gilia* sequenced to date. A close relationship among these three species was first formally recognized by Grant & Grant (1954). However, the great divergence between this lineage and the true gilia clade has not been previously considered. We are currently further investigating the affinities of the *G. splendens* group because its placement as sister to the *Allophyllum* and true gilia clades in the *mat*K analysis is only weakly supported (Fig. 6).

Finally, this analysis of *mat*K sequences recognizes *G. hutchinsifolia* and *G. leptomeria* (both of section *Giliandra*) as a well-supported clade that is also well separated from other gilias on the most parsimonious tree (Fig. 6). A clade including *G. hutchinsifolia* is similarly well removed from other gilias in analyses of ITS sequences (Fig. 8). Furthermore, both *mat*K and ITS sequences concur in placing *G. hutchinsifolia* and related species as sister to *Eriastrum*, *Langloisia*, and *Ipomopsis* (Fig. 6), although this relationship is not strongly supported in either analysis.

Polyploid origin of Saxifraga osloensis. The ability of *mat*K sequences to reveal even fine-scale relationships in taxonomic groups is illustrated by an example from the genus *Saxifraga*. *Saxifraga osloensis* is a tetraploid of evolutionary interest because it has been proposed by some as the only saxifrage that can confidently be considered an alloploid, although its parentage is controversial (reviewed in Webb & Gornall, 1989). Furthermore, it is also considered to be a classic example of a species of postglacial allopolyploid origin. Knaben (1954) hypothesized that *S. osloensis* is derived from two closely related diploid species, *S. tridactylites* and *S. adscendens*. C. Brochmann (pers. comm.), in contrast, has suggested that *S. osloensis* may be an autopolyploid derived from only *S. tridactylites*. Webb & Gornall (1989) note, however, that morphologically, *S. osloensis* more closely resembles *S. adscendens*. A comparison of only 750 bp of *mat*K sequence shows that *S. osloensis* is identical in sequence to *S. adscendens*, and the two species differ from *S. tridactylites* by 11 nucleotide differences. Thus, either *S. osloensis* is an autoploid derived from *S. adscendens*, or if an alloploid, *S. adscendens* was the maternal parent.

VARIABILITY

Indels. The Saxifragaceae s. s. and *Gilia* data sets suggest that a *mat*K sequence matrix with enough taxonomic breadth to show informative base substitutions is also likely to contain indels. For example, the 45-taxon Saxifragaceae s. s. *mat*K sequence matrix contains 10 indels (Fig. 2; Appendix 2), the 31-taxon *Gilia mat*K sequence matrix contains four indels (Fig. 6, Appendix 2), a 76-taxon Apiaceae–Pittosporaceae *mat*K sequence matrix contains 12 indels (Plunkett, 1994), and a seven-taxon Cupressaceae *mat*K sequence matrix contains two indels (P. Gadek and C. Quinn, pers. comm.). All of these indels are small, ranging in size from 3 to 9 bp. Because apparently identical indels may have multiple origins in unrelated taxa (Golenberg et al., 1993), we have not appended these indels as additional characters in the data matrices. However, only one of the 16 indels observed in the above matrices (indel E in the Saxifragaceae; Fig. 2, Appendix 2) appears homoplasious when mapped on trees derived from analyses

of base substitutions alone. Furthermore, given the poor resolution in the placement of *Astilbe* and the possibility of long-branch attraction in the placement of *Saxifraga mertensiana* on the most parsimonious trees for Saxifragaceae s. s. (Fig. 2), even this indel may have had a single origin. Although it has been our experience with *mat*K that indels can usually be aligned with a high degree of confidence if the reading frame is taken into consideration, we recognize that uncertainties in the alignment of indels is a potential source of error in analyses (Ritland & Clegg, 1987). The amount of error is likely to be minimal, however, given that few bases are involved in indels relative to the number of potentially informative characters that are unambiguously aligned.

Base substitutions. Most base substitutions in the Saxifragaceae s. s. and *Gilia mat*K data sets are hierarchically structured as evidenced by the randomization test of Archie (1989a). Furthermore, the homoplasy observed in these large *mat*K data matrices is not only modest (Table 2), but is also evenly distributed on the most parsimonious trees (data not shown). Together with the strong bootstrap support and high decay values for many of the clades present in the phylogenetic trees for Saxifragaceae s. s. and *Gilia* (Figs. 2 and 6, respectively), these observations suggest that both matrices have been constructed within an appropriate range of taxonomic hierarchy. Thus, although a minimum of 85% (in Saxifragaceae s. s.) and 73% (in *Gilia*) of the potentially informative characters in these *mat*K sequence matrices have experienced multiple hits (based on the number of potentially informative characters with 3 or 4 character states), most of these multiple hits convey phylogenetic information. Whereas multiple substitutions per site may be problematic in *mat*K analyses at broad taxonomic levels, they do not appear to be unduly problematic in analyses of intergeneric and interspecific relationships.

PCR AND SEQUENCING PRIMERS

To stimulate the use of *mat*K sequence data in other groups, we have tried to assess the broad applicability of the PCR and sequencing primers described herein. The *trn*K coding regions are very conserved among angiosperms, and PCR primers *trn*K-2R and *trn*K-3914F (Table 1) have been used by ourselves and others to amplify *mat*K from dicots such as Annonaceae, Apiaceae, Asteraceae, Balsaminaceae, Brassicaceae, Cornaceae, Ericaceae, Grossulariaceae, Lauraceae, Magnoliaceae, Malpighiaceae, Malvaceae, Polemoniaceae, Rhamnaceae, Rosaceae, Sarraceniaceae and Saxifragaceae. These primers have also been used to amplify *mat*K from monocots such as Iridaceae, Juncaceae, and Orchidaceae, although the latter family requires twice the standardly employed amount of $MgCl_2$ to achieve successful amplification via PCR (M. Chase, pers. comm.). For routine sequencing of monocots, however, it may be desirable to use Learn's original *trn*K-3914F primer (Table 1) that provides even greater homology to the 5′ *trn*K exon in these plants. Outside of angiosperms, primers *trn*K-3914F and *trn*K-2R have been used successfully to amplify *mat*K from Cupressaceae (P. Gadek & C. Quinn, pers. comm.). The similarity of these amplification primers to published *trn*K sequences of *Pinus* (Lidholm & Gustafsson, 1991) is high for *trn*K-3914F, but somewhat lower for *trn*K-2R. It may therefore be desirable to modify *trn*K-2R for routine use in conifers. Both *trn*K-3914F and *trn*K-2R also exhibit high similarity to a published sequence for *Marchantia* (Umesono et al., 1988) and may be useful for amplifying *mat*K from bryophytes as well. The PCR amplification primers *rps*16-4547F and *psb*A-R are useful as alternatives to primers *trn*K-3914F and *trn*K-2R, respectively, in some circumstances, but these primers may not prove as widely applicable (Fig. 1; Table 1). For example, *rps*16 (Fig. 1) is not present in *Marchantia* (Umesono et al., 1988), and *psb*A (Fig. 1) is duplicated between *rps*16 and the 5′ *trn*K exon in at least some species of *Pinus* (Lidholm & Gustafsson, 1991). More recently, primers *trn*K-253F, *trn*K-710F, and *trn*K-2000R have been used in various combinations with the standard PCR primers described above to obtain shorter fragments that contain *mat*K from taxa in Apicaceae (Plunkett, 1994) and *Saxifraga* that otherwise have yielded poor *mat*K PCR products. For sequencing, primers *mat*K-1235R, *mat*K-1470R, and *mat*K-1412F (Table 1) appear fairly conserved, at least among dicots, and enable the generation of sequences of approximately 1100 contiguous bp of *mat*K beginning at the 5′ end. We have used these sequencing primers successfully in Apiaceae, Brassicaceae, Cornaceae, Ericaceae, Grossulariaceae, Polemoniaceae, and Saxifragaceae. Other sequencing primers are less conserved, have not been broadly tested, or, in the case of primer *mat*K-1168R, the 3′ nine nucleotides are duplicated within the annealing site of *mat*K-1470R, and have annealed at both sites in some Saxifragaceae and Polemoniaceae, but not Apiaceae (G. Plunkett, pers. comm.). Of the sequencing primers we have used in angiosperms, *mat*K-1470R has also worked in the Cupressaceae

(P. Gadek & C. Quinn, pers. comm.) and thus appears to be a good choice for initial sequencing of *mat*K in diverse plant groups. Given the rapid rate of *mat*K sequence evolution, we have found that at least some primers will need to be designed specifically for a given group in order to sequence all of *mat*K. For example, whereas *mat*K-1168R, *mat*K-1235R, and *mat*K-1470R worked well in nearly all members of Saxifragaceae s. s. sequenced for this study, comparative sequencing within just the large genus *Saxifraga* has required the synthesis of three additional sequencing primers (*trn*K 710F, *mat*K-1176F, and *mat*K-1412R; D. Soltis, unpublished). The need to design group specific sequencing primers is likely to be true of any gene as large as *mat*K, however, that also evolves at a rate useful for intrafamilial and intrageneric phylogenetic reconstruction.

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APPENDIX 1. Species included in the *Gilia matK* sequence matrix (referenced to Fig. 6), the Saxifragaceae s.s. *matK* sequence matrix (referenced to Fig. 2), the three Saxifragaceae s.s. matrices composed of combined cpDNA restriction sites (r. s.) and *matK* and *rbcL* sequences (matrix-1 is referenced to Fig. 3; matrix-2 is referenced to Fig. 4; and matrix-3 is referenced to Fig. 5), and the comparison between *rbcL* and *matK* sequences for 25 species (referenced to Table 3). GenBank accession numbers are reported for all *matK* and *rbcL* sequences, whereas cpDNA restriction site data are located in Soltis et al. (1991) and Soltis et al. (1993).

Family Species	Voucher/citation	Data type	GenBank	Figure/Table
GROSSULARIACEAE				
Ribes aureum Pursh	*Soltis & Soltis 2220*, WS	*mat*K	L34153	2, 3, 4, 5/3
	Morgan & Soltis, 1993	*rbc*L	L11204	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Tetracarpaea tasmanica Hook. f.	*Jordon s.n.*, HO	*mat*K	L34154	2, 5/3
	Morgan & Soltis, 1993	*rbc*L	L11207	5/3
POLEMONIACEAE				
Allophyllum gilioides (Benth.) Grant & Grant	*Johnson 92012*, WS	*mat*K	L34176	6
Bonplandia geminiflora Cav.	*Patterson s.n.*, WS	*mat*K	L34179	6
Cantua pyrifolia Juss.	*Patterson s.n.*, WS	*mat*K	L34180	6
Collomia linearis Nutt.	*Johnson 92045*, WS	*mat*K	L34188	6
Eriastrum densifolium (Benth.) Mason	*Johnson 92090*, WS	*mat*K	L34184	6
Gilia achilleifolia Benth.	*Schultz s.n.*, WS	*mat*K	L34175	6
Gilia angelensis Grant	*Johnson 92013*, WS	*mat*K	L34177	6
Gilia australis (Mason & Grant) Grant & Grant	*Johnson 92021*, WS	*mat*K	L34178	6
Gilia capillaris Kellogg	*Johnson 93104*, WS	*mat*K	L34181	6
Gilia capitata Sims	*Johnson 92015*, WS	*mat*K	L34182	6
Gilia caruifolia Abrams	*Johnson 93096*, WS	*mat*K	L34183	6
Gilia filiformis Gray	*Johnson 93015*, WS	*mat*K	L34185	6
Gilia hutchinsifolia Rydb.	*Johnson 93069*, WS	*mat*K	L34186	6
Gilia inconspicua (Smith) Sweet	*R. Johnson 149*, WS	*mat*K	L34187	6
Gilia leptalea (Gray) Greene	*Patterson s.n.*, WS	*mat*K	L34195	6
Gilia leptantha Parish	*Schultz 52503*, WS	*mat*K	L34197	6
Gilia leptomeria Gray	*Johnson 93008*, WS	*mat*K	L34196	6
Gilia ochroleuca Jones	*Johnson 92022*, WS	*mat*K	L34189	6
Gilia scopulorum Jones	*R. Johnson 304*, WS	*mat*K	L34190	6
Gilia sinuata Benth.	*Johnson 92004*, WS	*mat*K	L34198	6
Gilia splendens Dougl. ex Lindl.	*Johnson 92093*, WS	*mat*K	L34191	6
Gilia stellata Heller	*Johnson 93059*, WS	*mat*K	L34199	6
Gilia tenerrima Gray	*Johnson 93103*, WS	*mat*K	L34192	6
Ipomopsis aggregata (Pursh) Grant	*Johnson 92100*, WS	*mat*K	L34193	6
Ipomopsis congesta (Hook.) Grant	*R. Johnson 166*, WS	*mat*K	L34200	6
Ipomopsis polycladon (Torrey) Grant	*Johnson 93068*, WS	*mat*K	L34194	6
Langloisia setosissima (Torr. & Gray) Greene	*Johnson 93074*, WS	*mat*K	L34201	6
Navarretia intertexta (Benth.) Hook.	*Glazner 9349*, WS	*mat*K	L34202	6
Phlox gracilis Greene	*Johnson 92046*, WS	*mat*K	L34203	6
Phlox hoodii Richardson	*Johnson 92001*, WS	*mat*K	L34205	6
Polemonium californicum Eastw.	*Johnson 93089*, WS	*mat*K	L34204	6
SAXIFRAGACEAE				
Astilbe japonica × Chinesensis	*Johnson s.n.*, WS	*mat*K	L34114	2, 4
Astilbe taquetii (Léveillé) Koidzumi	Morgan & Soltis, 1993	*rbc*L	L11173	4, 5
	Soltis et al., 1990	r. s.	—	4, 5
Astilboides tabularis (Hemsl.) Engl.	Univ. Oslo Bot. Gard., Norway, O	*mat*K	L34115	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06207	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5

Appendix 1. Continued.

Family Species	Voucher/citation	Data type	GenBank	Figure/Table
Bensoniella oregona (Abrams & Bacig.) Morton	*Soltis & Soltis, s.n.*, WS	*mat*K	L34112	2, 3, 4, 5/3
	Soltis & Soltis, s.n., WS	*rbc*L	L34072	3, 4, 5/3
	Soltis et al., 1991	r. s.	—	3, 4, 5
Bergenia cordifolia (Haw.) A. Br.	Komarov Bot. Inst., Leningrad, Russia, LE	*mat*K	L34116	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06208	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Bolandra oregana Wats.	*Grable 11587*, WS	*mat*K	L34117	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06209	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Boykinia occidentalis Torr. & Gray	*Grable 11636*, WS	*mat*K	L34118	2, 5
	Soltis et al., 1993	r. s.	—	5
Boykinia rotundifolia Parry	*Gornal 0101*, UBC	*mat*K	L34119	2, 3, 4, 5/3
	Morgan & Soltis, 1993	*rbc*L	L11175	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Chrysosplenium americanum Schw.	Soltis et al., 1993	r. s.	—	4
Chrysosplenium iowense Rydb.	*Wendel s.n.*, ISC	*mat*K	L34120	2, 4, 5
	Johnson & Soltis, 1994	*rbc*L	L19935	4, 5
Chrysosplenium tetrandrum (Lund) Fries	*Straly 6205*, UBC	*mat*K	L34121	2
Conimitella williamsii (Eaton) Rydb.	*Soltis & Soltis 1608*, WS	*mat*K	L34122	2, 5
	Soltis et al., 1991	r. s.	—	5
Darmera peltata (Torr.) Voss	*Soltis & Soltis 2083*, WS	*mat*K	L34123	2, 3, 4, 5/3
	Morgan & Soltis, 1993	*rbc*L	L11180	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Elmera racemosa (Wats.) Rydb.	*Soltis & Soltis 2234*, WS	*mat*K	L34124	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06210	3, 4, 5/3
	Soltis et al., 1991	r. s.	—	3, 4, 5
Heuchera hirsutissima Rosend., Butt & Lak.	*Wallace s.n.*, RSA	*mat*K	L34125	2, 5
	Soltis et al., 1991	r. s.	—	5
Heuchera micrantha Dougl.	*Soltis & Soltis 1949*, WS	*mat*K	L34126	2, 3, 4, 5/3
	Soltis et al., 1990	*rbc*L	L01925	3, 4, 5/3
	Soltis et al., 1991	r. s.	—	3, 4, 5
Heuchera rubescens Torr.	Soltis et al., 1991	*mat*K	L34127	2, 5
	Soltis et al., 1991	r. s.	—	5
Jepsonia parryi (Torr.) Small	*Rieseberg* 1110, RSA	*mat*K	L34128	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06211	3, 4, 5/3
	Soltis et al., 1993	r. s.		3, 4, 5
Leptarrhena pyrolifolia (D. Don) R. Br.	*Soltis & Soltis 2237*, WS	*mat*K	L34129	2, 3, 4, 5/3
	Morgan & Soltis, 1993	*rbc*L	L11191	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Lithophragma affine Gray	*Pellmyr & Thompson s.n.*, WS	*mat*K	L34130	2, 5
	Soltis et al., 1991	r. s.	—	5
Mitella caulescens Nutt.	*Soltis & Soltis 1881*, WS	*mat*K	L34131	2, 5
	Soltis et al., 1991	r. s.	–	5
Mitella diphylla L.	*Soltis & Soltis 1857*, WS	*mat*K	L34132	2, 5
	Soltis et al., 1991	r. s.	—	5
Mitella diversifolia Greene	*Soltis & Soltis 1910*, WS	*mat*K	L34133	2, 5
	Soltis et al., 1991	r. s.	—	5
Mitella nuda L.	*Johnson & Brunsfeld 1908*, WS	*mat*K	L34134 —	2, 5
	Soltis et al., 1991	r. s.		5
Mitella pentandra Hook.	*Grable 11432*, WS	*mat*K	L34135	2, 5
	Soltis et al., 1991	r. s.		5
Mitella stauropetala Piper	*Soltis & Soltis 1856*, WS	*mat*K	L34136	2, 5
	Soltis et al., 1991	r. s.	—	5

APPENDIX 1. Continued.

Family Species	Voucher/citation	Data type	GenBank	Figure/Table
Mukdenia rosii (Oliver) Koidzumi	*Soltis s.n.*, WS	*mat*K	L34137	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06212	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Peltoboykinia tellimoides (Maxim.) Hara	*Soltis s.n.*, WS	*mat*K	L34138	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06213	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Rodgersia pinnata Franch.	*Soltis s.n.*, WS	*mat*K	L34139	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06214	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Saxifraga cernua L.	Soltis et al., 1993	*mat*K	L34140	2, 5/3
	Soltis et al., 1993	*rbc*L	U06215	5/3
Saxifraga ferruginea Grah.	Soltis et al., 1993	*mat*K	L34141	2, 5
	Soltis et al., 1993	r. s.	—	5
Saxifraga integrifolia Hook.	*Soltis & Soltis 2253*, WS	*mat*K	L20131	2, 5/3
	Morgan & Soltis, 1993	*rbc*L	L01953	5/3
Saxifraga mertensiana Bong.	*Grable 11586*, WS	*mat*K	L34142	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06216	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Saxifraga oppositifolia L.	Soltis et al., 1993	*mat*K	L34143	2, 5/3
	Soltis et al., 1993	*rbc*L	U06217	5/3
Saxifraga punctata L. [= *S. nelsoniana* D. Don]	Soltis et al., 1993	*mat*K	L34144	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06218	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Suksdorfia ranunculifolia (Hook.) Engl.	*Soltis & Soltis 2308*, WS	*mat*K	L34145	2, 5
	Soltis et al., 1993	r. s.	—	5
Suksdorfia violacea A. Gray	*Soltis & Soltis 2309*, WS	*mat*K	L34146	2, 5
	Soltis et al., 1993	r. s.	—	5
Sullivantia oregana Wats.	Soltis et al., 1993	*mat*K	L34113	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06219	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Sullivantia sullivantii (Torr. & Gray) Britt.	*Quackenbush s.n.*, WS	*mat*K	L20130	2
Tanakaea radicans Franch.	Univ. Brit. Columbia Bot. Gard., UBC	*mat*K	L34147	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06220	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Telesonix heucheriformis Rydb.	*Wolf 151*, WS	*mat*K	L34148	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06221	3, 4, 5/3
	Soltis et al., 1993	r. s.	—	3, 4, 5
Tellima grandiflora (Pursh) Dougl. [= "northern" type]	*Soltis & Soltis 2113*, WS	*mat*K	L34149	2, 5
	Soltis et al., 1993	r. s.	—	5
Tellima grandiflora (Pursh) Dougl. [= "southern" type]	*Soltis & Soltis 2119*, WS	*mat*K	L34150	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06222	3, 4, 5/3
	Soltis et al., 1991	r. s.	—	3, 4, 5
Tiarella trifoliata L.	*Ness 533*, WS	*mat*K	L34151	2, 5
	Soltis et al., 1991	r. s.	—	5
Tolmiea menziesii (Pursh) Torr. & Gray	*Soltis & Soltis 1903*, WS	*mat*K	L34152	2, 3, 4, 5/3
	Soltis et al., 1993	*rbc*L	U06223	3, 4, 5/3
	Soltis et al., 1991	r. s.	—	3, 4, 5

APPENDIX 2. Insertion/deletion events (indels) observed in *mat*K sequence matrices following alignment. Indels are labeled alphabetically (A–D for *Gilia* and A–J for Saxifragaceae); these labels correspond to those mapped on the most parsimonious trees (Fig. 6 for *Gilia* and Fig. 2 for Saxifragaceae). Dashes represent missing bases associated with indels. Dots in the sequence below the reference taxon (***Gilia stellata*** for ***Gilia*** and ***Sullivantia oregana*** for Saxifragaceae) indicate that the same nucleotide present in the reference taxon is also present in the species containing the indel (note that all species in which an indel occurs are listed, although the sequence is given for only one of these species when more than one species possesses the same indel). P/A represents the number of species with nucleotides involved in indels present and absent, respectively. RN, the reference nucleotide, is the position of the last sequential nucleotide preceding the indel in unaligned sequences of the reference taxon for each data set.

Data set Indel	P/A	Representative sp.	RN	Sequence region
Gilia				
A	30A	*Gilia stellata*	270	CAAAATCACATT TTTGGGCACAAC ------ACGAAT TTGTATTATCAA
	1P	*Polemonium californ.*		.... AACAAC...... ....C...
B	30P	*Gilia stellata*	378	GAGTTAGTCAAA TCTCATAATTTA CGATCAATTCAT TCAATATTTCCT
	1A	*Bonplandia geminifl.*		A.A......... ...--------- ....
C	26P	*Gilia stellata*	573	CACGAATATCGT AATTGGAATAAT ATTATTACTACA AAAAAATCTAGT
	5A	*Gillia leptalea*		.......G.... ....--- ---......... C
		Gilia capillaris		
		Allophyllum gilioides		
		Collomia linearis		
		Navarretia intertexta		
D	30A	*Gilia stellata*	798	GAAGTATTTATT AAAGATCTTCAA ---GATATTCTA GGTTTGTTCAAG
	1P	*Cantua pyrifolia*		..........C. .... TCGTCG...... ..G........A
Saxifragaceae				
A	44P	*Sullivantia oregana*	105	TATGCACTTGCT CATGATCATGTT TTAAATAGATCC ATTTTGTTGGAT
	1A	*Tetracarpaea tasman.*		.... T........--- ------.....A TG.........A
B	43A	*Sullivantia oregana*	141	ATTTTGTTGGAT AATTTTGGT--- TATGACAATAAA TCCAGTTCAATA
	2P	*Chrysosplenium iowe.*		.C...T...A.. ...A.....GGT ...T........ ....C..
		Chrysosplenium teta.		
C	44P	*Sullivantia oregana*	237	ATTTCTGCTAAT TATTCGAACAAA AATAAATTTTTG GGGCATAACAAG
	1A	*Suksdorfia ranuncul.*		.... G........... ---------... ....
D	42A	*Sullivantia oregana*	276	AAGAATTTCGAT TCTCAA------ ATGATATCAGAG GGATTTGCAGTT
	3P	*Saxifraga integrifo.*		........A... .A....AATAAA C
		Saxifraga punctata		
		Saxifraga ferrugine.		
E	42A	*Sullivantia oregana*	354	TCTTCCTTAGAA AGGAAAGAA--- ATAGTAAAATCT CATAATTTACGA
	3P	*Saxifraga cernua*		......G..... ....GAA ....G....... ....
		Saxifraga oppositif.		
		Astilbe jap. × chin.		
F	44P	*Sullivantia oregana*	366	GAAAGGAAAGAA ATAGTAAAATCT ------CATAAT TTACGATCAATT
	1A	*Saxifraga mertensia.*		.....A...... .....C.....G CAAAAT..A... ....C...
G	40P	*Sullivantia oregana*	366	GAAAGGAAAGAA ATAGTAAAATCT CATAATTTACGA TCAATTCATTCA
	5A	*Heuchera micrantha*		....A....... T........... -----....... ....
		Conimitella willia.		
		Mitella diversifol.		
		Mitella stauropeta.		
		Tellima grandif. S.		
H	43A	*Sullivantia oregana*	549	TTTCTCTATGAG TAT------CAG AGTTGGAATAGT CTTATTACCCCA
	2P	*Chrysosplenium iowe.*		.....T...... ...TATATT.C .A..TA..C.A. ....
		Chrysosplenium teta.		
I	44P	*Sullivantia oregana*	573	AGTTGGAATAGT CTTATTACCCCA ACTCCAAAGAAA TCCATTTCCATT
	1A	*Tetracarpaea tasman.*		.A.......... ....T--- ---......... ....T...
J	43P	*Sullivantia oregana*	591	CCAACTCCAAAG AAATCCATTTCC ATTGTTTCACAA AGGAATCAAAGA
	2A	*Leptarrhena pyrolif.*		...........T ......------ ....A.. ....
		Tanakaea radicans		

EVIDENCE FOR THE POLYPHYLY OF THE SCROPHULARIACEAE BASED ON CHLOROPLAST *rbc*L AND *ndh*F SEQUENCES[1]

Richard G. Olmstead[2] *and Patrick A. Reeves*[3]

ABSTRACT

Data from the chloroplast genes *rbc*L and *ndh*F, totaling more than 3500 base pairs of DNA sequence, were used to examine the monophyly of the Scrophulariaceae, including several groups that have been suggested to belong to or to be derived from the Scrophulariaceae. Thirty-two taxa representing the Lamiales s.l. and outgroups were sampled and each of the sets of gene sequences was analyzed separately and in combination. Results indicate that two distinct clades composed of elements of the traditional Scrophulariaceae exist and that a monophyletic Scrophulariaceae, even one liberally circumscribed to include several small families, cannot be supported by these data. One group, designated "scroph I," includes *Verbascum, Celsia, Selago, Scrophularia, Buddleja,* and *Nicodemia.* A second group, "scroph II," includes *Antirrhinum, Digitalis, Veronica,* and the Plantaginaceae, Callitrichaceae, and Hippuridaceae. *Schlegelia* and *Paulownia,* often assigned either to the Scrophulariaceae or Bignoniaceae, do not appear with either family.

The Scrophulariaceae are a well-known family to temperate botanists and one for which a "gestalt" recognition serves the scientist well. Members of the family are generally recognized by their usually herbaceous habit, their typically bilaterally symmetric, tubular flowers, and their many-seeded capsular fruits. Taxonomists learn the exceptions to the "usual," such as the woody *Paulownia* trees and the nearly actinomorphic flowers of *Verbascum,* and learn the characters that identify related groups: the winged seeds lacking endosperm of the Bignoniaceae, the achlorophyllous parasites of the Orobanchaceae, the wind-pollinated Plantaginaceae, the reduced ovule number in the Labiatae and Verbenaceae, and the inflorescence bracts and explosive capsules of the Acanthaceae. However, the characters by which we recognize plants as scrophs cannot be labeled shared derived traits, or synapomorphies, leaving open the possibility that the family is not monophyletic.

The taxonomic history of the Scrophulariaceae reflects the problem of describing a natural group. Most recent angiosperm classifications (e.g., Cronquist, 1981; Takhtajan, 1987; Thorne, 1992) have recognized a suprafamilial group centered around the Scrophulariaceae and containing approximately 12 (e.g., Scrophulariales of Cronquist, 1981) to 15 (e.g., Scrophulariinae of Thorne, 1992) families. Some of the more clearly defined families in the group are suggested to be connected to the Scrophulariaceae by intermediate genera (e.g., *Nelsonia* connecting with the Acanthaceae or *Paulownia* and *Schlegelia* connecting with the Bignoniaceae), whereas other families (e.g., Orobanchaceae, Plantaginaceae, Lentibulariaceae) "may logically be considered to be specialized derivatives of the Scrophulariaceae" (Cronquist, 1981). The Scrophulariaceae, as circumscribed in the abovementioned treatments, usually have the largest number of species of any family in the group (e.g., 3000, Thorne, 1992). Most recent treatments of the Scrophulariaceae and related families are derived from the 19th century treatment of Bentham & Hooker (Bentham, 1876), who maintained separate families for the Lentibulariaceae, Orobanchaceae, Plantaginaceae, and Selaginaceae. Wettstein (1895) included the Selaginaceae as a tribe in the Scrophulariaceae. Hallier (1903), in recognition of the apparently derived nature of the Lentibulariaceae, Orobanchaceae, Plantaginaceae,

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and Selaginaceae relative to the "core" Scrophulariaceae, included them in the Scrophulariaceae. However, since Hallier (1903), classifications (e.g., Cronquist, 1981; Takhtajan, 1987; Thorne, 1992) have followed Wettstein (1895) in maintaining as distinct families the Lentibulariaceae, Orobanchaceae (except Takhtajan, 1987; Thorne, 1992), and Plantaginaceae. Infrafamilial classifications have recognized three subfamilies, Pseudosolaneae (primarily tribe Verbasceae), Antirrhinoideae, and Rhinanthoideae (e.g., Wettstein, 1895), or two subfamilies with Pseudosolaneae subsumed by Antirrhinoideae (e.g., Thieret, 1967), or, when Orobanchaceae is included, it is maintained as a third subfamily (Takhtajan, 1987; Thorne, 1992).

The placement of several genera putatively related to Scrophulariaceae, including a few monogeneric families (e.g., Callitrichaceae, Hippuridaceae, Hydrostachyaceae), has been disputed in angiosperm classifications. *Paulownia*, a large woody tree similar in habit, as well as flower, inflorescence, and leaf morphology, to *Catalpa* (Bignoniaceae), was placed in the Scrophulariaceae by Bentham and Hooker (Bentham, 1876) and Wettstein (1895), but was placed in the Bignoniaceae by Takhtajan (1980) and Cronquist (1981). However, Armstrong (1985) marshaled the evidence in favor of its placement in the Scrophulariaceae, and subsequent classifications have placed it there (e.g., Takhtajan, 1987; Thorne, 1992). *Schlegelia* and the related genera *Gibsoniothamnus* and *Synapsis* similarly have been bounced back and forth between the same two families, while not being considered closely related to *Paulownia*. Bentham and Hooker included *Schlegelia* in the Bignoniaceae, and this placement has been followed in the monograph of the family by Gentry (1980) and in Cronquist (1981) and Takhtajan (1987). Armstrong (1985) also considered *Schlegelia* and related genera and found that they did not fit with the Bignoniaceae (e.g., they have seeds with endosperm) and fit better within the Scrophulariaceae, although they are distinctive within that family. Thorne (1992) followed Armstrong (1985) by including the Schlegelieae in the Scrophulariaceae.

The Selagineae, a group of several genera and 100–200 species mostly native to South Africa and Madagascar, are distinguished by the presence of a single ovule in each of the two locules in the ovary (Thieret, 1967). This group was maintained as a distinct family by Bentham and Hooker (Bentham, 1876), but included in the Scrophulariaceae by Wettstein (1895) and most recent treatments (e.g., Takhtajan, 1987; Thorne, 1992). However, Cronquist (1981) placed the Selagineae in the Globulariaceae. The Globulariaceae are a small group of two genera and approximately 30 species (Thorne, 1992) that traditionally have been considered a distinct family, but also have been described as a tribe within the Scrophulariaceae (Barringer, 1993).

Aquatic angiosperms traditionally have been difficult to classify, and several aquatics have been associated with the Scrophulariales and related orders. The Lentibulariaceae are aquatic or semiaquatic and insectivorous, but retain showy insect-pollinated flowers that suggest a relationship with the Scrophulariaceae and have been classified close to that family or included within it (e.g., Hallier, 1903). However, the Callitrichaceae, Hippuridaceae, and Hydrostachyaceae, each a monogeneric family, all have very reduced floral morphology associated with either wind pollination (*Hippuris, Hydrostachys*) or water pollination (*Callitriche*), and their taxonomic placement has varied dramatically. Based primarily on embryological characters (e.g., the presence of unitegmic and tenuinucellate ovules) and evidence from floral development, these taxa have been placed in the Asteridae near the Scrophulariales or Lamiales (evidence reviewed in Wagenitz, 1992). Cronquist (1981) placed the three families together in the order Callitrichales, but this treatment has not been accepted widely (Wagenitz, 1992).

Root parasitism is a well-developed habit in the Scrophulariaceae, and the existence of green (photosynthetic) hemiparasites, such as *Orthocarpus, Pedicularis*, and *Striga*, is presumed to indicate a link to the achlorophyllous holoparasites of the Orobanchaceae. The dramatically reduced chloroplast genome in the Orobanchaceae (dePamphilis & Palmer, 1990), involving the loss of many genes involved with photosynthesis and other functions (Wolfe et al., 1992), has precluded the inclusion of representatives of the group in chloroplast DNA (cpDNA) studies involving either restriction site mapping of the entire genome or sequencing of *rbc*L. However, evidence from the cpDNA inverted repeat region (C. dePamphilis, pers. comm.) and DNA sequencing of the nuclear 18S rDNA (A. Colwell, pers. comm.) provide support linking the Orobanchaceae to the hemiparasites of the Scrophulariaceae.

A picture of phylogenetic relationships in the Asteridae has begun to emerge from studies of cpDNA, in which a monophyletic group, including the Scrophulariaceae and related families and provisionally designated the Lamiales s.l., has been identified (Downie & Palmer, 1992; Olmstead et al., 1992, 1993a). Initial studies of the chloroplast

gene *rbc*L were aimed at the identification and circumscription of major lineages within the Asteridae (Bremer et al., 1994; Olmstead et al., 1992, 1993a). Consequently, sampling of many families was inadequate to test hypotheses of monophyly at the family level. For previous cpDNA sequencing studies within the Lamiales s.l., sampling in the Scrophulariaceae has been limited to *Antirrhinum* and *Digitalis* (Olmstead et al., 1993a). Of the disputed genera and closely related families discussed above, only the Lentibulariaceae (Chase et al., 1993; Olmstead et al., 1993a) and Callitrichaceae (Olmstead et al., 1992, 1993a) have been sampled previously. Based on these analyses, the Lentibulariaceae were not closely related to the Scrophulariaceae, whereas *Callitriche*, usually placed more closely to the Labiatae (e.g., Wagenitz, 1992), forms a monophyletic group with *Antirrhinum* and *Digitalis* (Olmstead et al., 1993a).

In addition to the broad studies that have identified the Lamiales s.l. clade, other studies have addressed particular families within the Lamiales s.l. using cpDNA sequence data. Scotland et al. (1995), using *rbc*L and *ndh*F sequences, have identified a monophyletic Acanthaceae, which includes the often segregated Thunbergiaceae and Nelsoniaceae. Wagstaff & Olmstead (unpublished), using *rbc*L sequences, have confirmed a new division between the Labiatae and Verbenaceae, following Cantino's (1992) morphological cladistic study of the Labiatae. A monophyletic Labiatae has been established (Cantino et al., 1992), which includes much of the former Verbenaceae, leaving a reduced and apparently monophyletic Verbenaceae comprising only the former subfamily Verbenoideae. Also, a monophyletic Bignoniaceae has been circumscribed on the basis of *rbc*L and *ndh*F sequences (R. Spangler & R. Olmstead, unpublished), and similar work is under way on the Gesneriaceae (J. Smith, pers. comm.).

The goals of this study are to explore the possibility that the Scrophulariaceae (e.g., sensu Thorne, 1992) are not a monophyletic group and to examine the placement of several of the disputed genera and small families putatively allied with the Scrophulariaceae. The goal is not to establish an infrafamilial classification, because substantial doubt exists regarding whether the group is monophyletic in the first place. Attempts to discover an infrafamilial phylogeny using cpDNA inverted repeat restriction site mapping have met with frustration, for example, due to the lack of evidence for a monophyletic Scrophulariaceae (C. dePamphilis, pers. comm.). However, these efforts have discovered many groups of related genera, which ultimately may bear recognition at some level in a revised classification of the Lamiales s.l.

Sequencing the chloroplast gene *rbc*L has proven to be a useful approach for identifying phylogenetic relationships among higher-order groups in the angiosperms (e.g., *Annals of the Missouri Botanical Garden* Vol. 80(3), 1993) and has been particularly helpful in identifying major lineages within the Asteridae (Olmstead et al., 1993a). However, the highly conserved nature of the gene results in relatively few informative phylogenetic characters at the infrafamilial level. Sequencing studies focusing on individual families, with sufficient sampling to examine infrafamilial relationships (Conti et al., 1993; Doebley et al., 1990; Kim et al., 1992; Olmstead & Sweere, 1994; Soltis et al., 1993), have made clear the need to sample larger amounts of sequence to acquire sufficient numbers of characters to gain resolution and to assure greater accuracy in phylogenetic reconstructions. For this study, which crosses family lines to examine infra- as well as interfamilial relationships, a longer and more rapidly evolving cpDNA gene, *ndh*F (Olmstead & Sweere, 1994), was chosen to complement the substantial data set of *rbc*L gene sequences already available for representatives of the Lamiales s.l. By sequencing both genes for all taxa included in this analysis, nearly 3500 base pairs (bp) of cpDNA are available for phylogenetic inference, relative to the approximately 1400 bp compared in studies of *rbc*L alone.

The gene *ndh*F in tobacco encodes a protein of 740 amino acids, which is suggested to be a subunit of an NADH dehydrogenase enzyme (undiscovered at present) in the chloroplast (Sugiura, 1992). It is located in the small single-copy (SSC) region of the chloroplast genome close to the junction with the inverted repeat (IR). The termination codon of *ndh*F is located 43 bp from the IR/SSC boundary in tobacco, and transcription proceeds toward the inverted repeat. A comparison of rice and tobacco sequences suggests that *ndh*F has a nucleotide substitution rate approximately two times higher than that of *rbc*L (Sugiura, 1989). In combination with the fact that *ndh*F is approximately 50% longer than *rbc*L, the two-fold higher substitution suggests a three-fold increase in cladistic characters. In a previous study of the Acanthaceae (Scotland et al., 1995), almost exactly three times the number of characters were derived from *ndh*F relative to *rbc*L (421 vs. 136, note that taxon sampling was slightly different for the two genes); however, in a study of the Solanaceae (Olmstead & Sweere, 1994) *ndh*F provided only 60% more characters than *rbc*L (100 vs. 63).

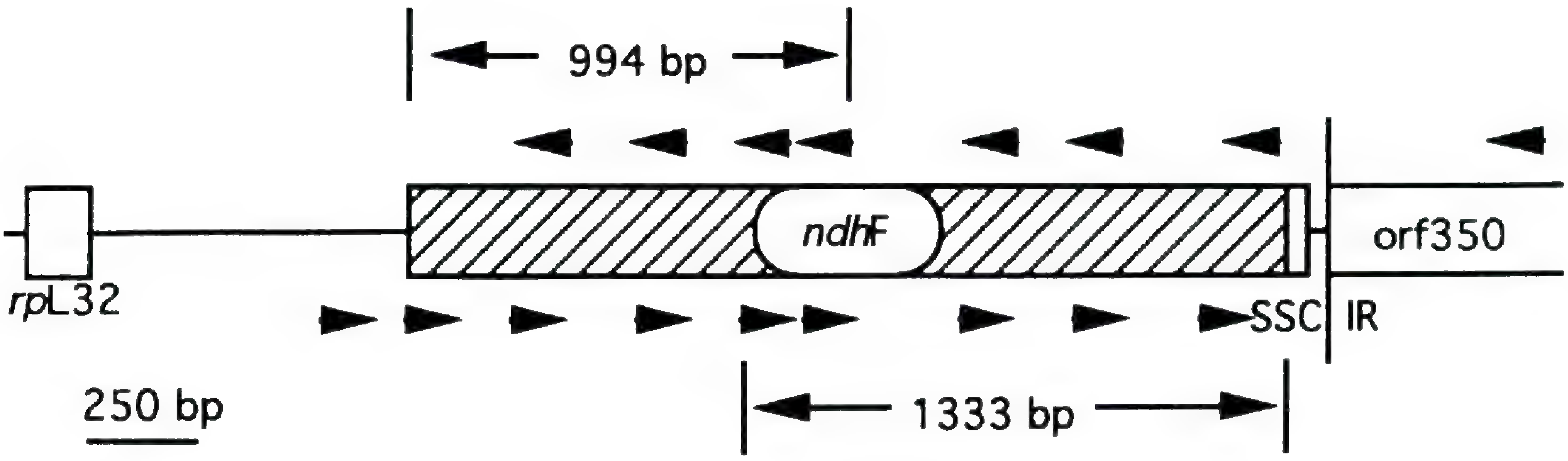

FIGURE 1. Map of *ndh*F and adjoining regions of cpDNA in tobacco. Boxes indicate reading frames; lines connecting boxes indicate noncoding DNA. Vertical bar at left end of orf350 indicates junction between inverted repeat (IR) and small single-copy region (SSC). Arrows indicate location of PCR and sequencing primers (primers located outside the coding region for *ndh*F supplied by R. Jansen, University of Texas). Cross-hatching in *ndh*F indicates portion sequenced. Overlapping regions marked above and below the map indicate the two fragments in which the gene was amplified.

MATERIALS AND METHODS

This study includes 29 representative species of the Lamiales s.l. and three outgroup species representing the most closely related orders; species names, voucher information, and GenBank accession numbers or prior publication references are given in Table 1. Eight tribes and both subfamilies Antirrhinoideae and Rhinanthoideae are represented (following Thieret, 1967), including six species classified as Scrophulariaceae by the most conservative treatments of the family: *Antirrhinum* (subfam. Antirrhinoideae, tribe Antirrhineae), *Celsia* (Antirrhinoideae, Verbasceae), *Scrophularia* (Antirrhinoideae, Scrophularieae), *Verbascum* (Antirrhinoideae, Verbasceae), *Digitalis* (Rhinanthoideae, Digitaleae), and *Veronica* (Rhinanthoideae, Veroniceae). Also included are disputed members *Paulownia* (Antirrhinoideae, Paulownieae), *Selago* (Antirrhinoideae, Selagineae), and *Schlegelia* (Antirrhinoideae, Schlegelieae), and representatives of three small putatively related families, Callitrichaceae, Hippuridaceae, and Plantaginaceae. Three additional species representing the Lentibulariaceae were included in an analysis of *rbc*L sequences with the 32 other species (results not shown). DNA sequences for the two genes were determined from the same plant accession for nearly all taxa. Exceptions include *Nicotiana* and *Barleria*, for which different accessions of the same species were used to determine the two gene sequences; *Thunbergia*, for which two different species were used, *T. usamberica* for *rbc*L and *T. alata* for *ndh*F; and *Verbena*, for which two different species were used, *V. bonariensis* for *rbc*L and *V. bracteata* for *ndh*F. Of the 64 sequences included in this study, 43 are not previously reported. It was determined during the course of this study that the previously published *rbc*L sequence of *Buddleja*, and the DNA accession from which it was derived, was mistakenly that of *Paulownia tomentosa* (cf. Bremer et al., 1994; Chase et al., 1993; Olmstead et al., 1993a; Scotland et al., 1995); the correct sequence for *Buddleja* is reported here.

Procedures for determining DNA sequences were as described in Olmstead & Sweere (1994). PCR amplification primers for *rbc*L are those described in Olmstead et al. (1992), with internal sequencing primers provided by G. Zurawski (DNAX Research Institute, Palo Alto, California). PCR amplification primers and internal sequencing primers for *ndh*F are described in Olmstead & Sweere (1994), with the exception that primers #1 and #2110R (forming the 5′ and 3′ ends of the amplified regions, respectively) were redesigned to match more exactly the homologous sequences for the Lamiales and were used to determine some sequences (Fig. 1). Redesigning the primers was made possible by sequencing through the ends of the gene in several species using primers located in flanking regions (primers kindly provided by R. Jansen, University of Texas). The new primer sequences are as follows (for others, see Olmstead & Sweere, 1994): #1—ATGGAACAGACATATCAATATG(C/G)GTGC and #2112R—CCC(C/T)A(C/G)ATATTTGATACCTTC(G/T)CC.

A total of 2135 bp of the *ndh*F gene was amplified in tobacco (somewhat more or less in other taxa depending on insertions and deletions) in two overlapping fragments of 994 bp and 1333 bp at the 5′ and 3′ ends of the gene respectively (Fig. 1). Approximately 97 bp at the 3′ end of the gene was not included. Preparation of DNA for sequenc-

TABLE 1. Sources of plant material, previously published sequences, and GenBank accession numbers for taxa included in this study (arrangement follows Thorne, 1992).

Family	Species	DNA Source/voucher[1]	*rbc*L	*ndh*F
Solanales				
Solanaceae	*Nicotiana tabacum* L.	Olmstead & Palmer, 1992	Lin et al., 1986	Olmstead et al., 1993b
Gentianales				
Gentianaceae	*Gentiana procera* Holm		Olmstead et al., 1993a	#L36400
Boraginales				
Boraginaceae	*Borago officinalis* L.		Olmstead et al., 1992	#L36393
Lamiales s.l.				
Acanthaceae	*Barleria prionitis* L.		Chase et al., 1993	Scotland et al., 1995
	Thunbergia usamberica Lindau		Chase et al., 1993	
	Thunbergia alata Bojer			Scotland et al., 1995
Bignoniaceae	*Catalpa* sp.		Olmstead et al., 1992	#L36397
	Martinella obovata (HBK) Bureau & K. Schum.	*Gentry 50277*	#L36444	#L36402
	Tabebuia heterophylla (A. de Candolle) Britton	Gentry (collection number unknown)	#L36451	#L36416
Buddlejaceae	*Buddleja davidii* Franch.		Olmstead et al., 1993a	#L36394
	Nicodemia diversifolia Tenore		Olmstead et al., 1993a	#L36405
Callitrichaceae	*Callitriche hermaphroditica* L.	*Philbrick 3022*	#L36441	#L36396
Gesneriaceae	*Nematanthus hirsutus* (Mart) Wiehler	Marie Selby B.G.	#L36446	#L36404
	Streptocarpus holstii Engl.		Olmstead et al., 1993a	#L36415
Hippuridaceae	*Hippuris vulgaris* L.	*Olmstead & Reeves 92-127*	#L36443	#L36401
Labiatae	*Ajuga reptans* L.		Wagstaff & Olmstead, unpublished	#L36391

TABLE 1. Continued.

Family	Species	DNA Source/voucher[1]	*rbc*L	*ndh*F
	Callicarpa dichotoma Koch		Olmstead et al., 1993a	#L36395
	Physostegia virginiana Benth.		Olmstead et al., 1993a	#L36407
Myoporaceae	*Myoporum mauritianum* A. de Candolle	*Olmstead 92-299*	#L36445	#L36403
Pedaliaceae	*Sesamum indicum* L.		Olmstead et al., 1993a	#L36413
Plantaginaceae	*Plantago lanceolata* L.	*Olmstead s.n.*	#L36454	#L36408
Scrophulariaceae	*Antirrhinum majus* L.		Olmstead et al., 1992	#L36392
	Celsia arcturus Jacq.	de Pamphilis (collection number unknown)	#L36442	#L36398
	Digitalis grandiflora Mill.		Olmstead et al,. 1993a	#L36399
	Paulownia tomentosa Steud.	*Olmstead 88-008*	#L36447	#L36406
	Selago thunbergii Choisy	*dePamphilis 90-21*	#L36450	#L36412
	Schlegelia parviflora (Oerst.) Monachino	*Gentry 14221*	#L36448	#L36410
	Scrophularia sp.	dePamphilis (collection number unknown)	#L36449	#L36411
	Verbascum thapsus L.	no voucher	#L36452	#L36417
	Veronica catenata Pennell	*Olmstead 92-144*	#L36453	#L36419
Verbenaceae	*Rhaphithamnus spinosus* (A. L. Juss.) Moldenke		Wagstaff & Olmstead, unpublished	#L36409
	Stachytarpheta dichotoma (Ruiz & Pav.) Vahl.		Wagstaff & Olmstead, unpublished	#L36414
	Verbena bonariensis L.		Olmstead et al., 1993a	
	Verbena bracteata Lagasca & Rodriguez	*Olmstead 92-131*		#L36418

[1] For voucher information on previously published sequences, see reference cited with sequence. References are cited in this column only when voucher information is cited in a reference other than the one in which the sequence is cited.

TABLE 2. Gaps in the sequence alignment for *ndh*F. Polarity (insertion vs. deletion) is relative to the results of the cladistic analysis of the nucleotide data. Coordinates are relative to the sequence for *Nicotiana tabacum.*

Insertion/deletion	Size (bp)	Coordinates[1]	Taxa
Insertion	6	657/658	*Nematanthus*
Insertion	6	1425/1426	*Selago*
Insertion/deletion	9	1443/1444	*Nicotiana*
Insertion	3	1443/1444	*Streptocarpus*
Deletion	42	1447-1488	*Callitriche*
Insertion	6	1467/1468	*Streptocarpus*
Insertion	6	1470/1471	*Selago*
Insertion/deletion	9	1471-1479	*Borago*
Insertion/deletion	9	1474-1482	*Gentiana*
Deletion	9	1477-1485	*Physostegia*
Insertion	12	1481/1482[2]	*Stachytarpheta*
Deletion	3	1486-1488	*Digitalis, Plantago, Veronica*
Deletion	6	1489-1494	*Veronica*
Deletion	6	1498-1503	*Barleria*
Insertion/deletion	9	1498-1506	*Gentiana*
Deletion	3	1501-1503	*Ajuga*
Deletion	9	1525-1533	*Verbena*
Insertion	6	1539/1540	all Lamiales s.l.; deleted in *Ajuga, Nicodemia*
Deletion	3	1564-1566	*Streptocarpus*
Insertion/deletion	6	1572/1573	*Gentiana*
Insertion	9	1695/1696	*Nematanthus, Plantago*
Insertion/deletion	6	1701/1702	*Gentiana, Plantago* (insertion)
Insertion/deletion	6	1702-1707	*Borago*
Deletion	6	1708-1713	*Barleria*
Insertion	6	1932/1933	*Streptocarpus*

[1] "/" indicates insertion between coordinates in *Nicotiana;* "-" range of nucleotides deleted relative to aligned *Nicotiana* sequence.

[2] Insertion not in frame with breaks between mRNA codons to enable identification of direct repeat as insertion.

ing proceeded by first amplifying each fragment with balanced primer concentrations to yield double-stranded DNA, then producing single-stranded DNA by a second round of PCR using a portion of the initial PCR product as template and a single primer (Kaltenboeck et al., 1992). Standard dideoxy sequencing was performed using Sequenase (U.S. Biochemical) and ^{32}P-labeled dATP. Each strand was sequenced for all taxa, requiring 12 sequencing reactions per *ndh*F sequence (Fig. 1).

Alignments of the sequences were determined by eye and, in the data set used for cladistic analysis, gaps in one species relative to any other were scored as missing data. The first 26 bp of the coding sequence of *rbc*L correspond to the 5′ PCR primer, and no gaps were identified through the first 1428 bp (the termination codon was beyond bp 1428 in all of the sequences), so 1402 bp of *rbc*L sequence were used for the phylogenetic analysis. Several gaps representing insertions, duplications, or deletions were observed in the *ndh*F sequences (Table 2). The first 23 bp of the coding sequence of *ndh*F correspond to the 5′ PCR primer, and the 3′ primer corresponds to bp 2110–2135 of the *Nicotiana tabacum* sequence. Inferred lengths of the *ndh*F genes sequenced for this study, including undetermined sequence downstream of the 3′ PCR primer, ranged in length from 2196 bp for *Callitriche heterophylla* to 2253 for *Nematanthus hirsutus.* The total aligned length including all gaps was 2167 bp.

The data were analyzed in three separate analyses: *rbc*L only, *ndh*F only, and both sequences combined. Parsimony analyses were conducted using PAUP version 3.1 (Swofford, 1993) with all changes weighted equally. For each analysis 100 random order entry replicate searches were conducted using the HEURISTIC search option with TBR branch swapping and with MULPARS "on" to save all equally most parsimonious trees. Results of each replicate were monitored to determine if multiple islands (Maddison, 1991) were discovered (as in Olmstead et al., 1993a). To assess the relative support for clades found in the combined analysis,

a bootstrap analysis was conducted with 100 replicates (Felsenstein, 1985). To examine possible circumscriptions of taxa that might represent a monophyletic Scrophulariaceae, four separate analyses were performed with each of the three data sets (each gene separately and the combined set) with various groups of taxa constrained to monophyly. The four constrained circumscriptions range from a strict definition of Scrophulariaceae, as found in many classifications, to a broad definition including many taxa of disputed family membership or belonging to small families inferred to be closely related to members of the Scrophulariaceae based on cpDNA studies. Following these constrained searches, a decay analysis (Donoghue et al., 1992) was performed on the combined data set, in which all trees were saved up to the length of the shortest tree in which a broadly defined monophyletic Scrophulariaceae was found. This last analysis was performed to determine how much structure remains in a tree based on these data if a monophyletic Scrophulariaceae is to be accepted. The data used in these analyses are available from the first author upon request, and sequences have been submitted to GenBank (Table 1).

Results

A total of 3569 bp of aligned sequence was used in this study, including 1402 bp (39%) of *rbc*L and 2167 aligned bp of *ndh*F. Of the 2167 bp of *ndh*F aligned sequence, 66 bp represent insertions or duplications unique to a single taxon or to two taxa and therefore are unable to provide any phylogenetic information, leaving 2101 bp of potentially useful aligned sequence. Of the 3569 total bp compared, 2196 bp (61.5%) were invariant, 640 bp (17.9%) were variable, but uninformative with respect to phylogeny, and 734 bp (20.6%) were phylogenetically informative. The *rbc*L sequences contributed 189 informative characters, or approximately 26% of the total while accounting for 40% of the useful aligned sequence, whereas *ndh*F sequences contributed 545 informative characters, or 74% of the total while accounting for only 60% of the useful aligned sequence. This 3 : 1 proportion in number of characters derived from *ndh*F relative to *rbc*L matches that found in the Acanthaceae (Scotland et al., 1995) and the prediction based on size and substitution rate of the two genes (Sugiura, 1989).

The presence of insertions and deletions (indels) in *ndh*F represents a significant difference relative to *rbc*L. In this study, 25 gaps in the sequence alignment are required to accommodate indels observed in the 32 sequences (Table 2). Of these, 21 are unique to an individual sequence (based on the results of the phylogenetic analysis, seven are insertions, eight are deletions, and six are among the outgroups and therefore unordered). Two gaps each apparently represent two independent insertions (inferred from the resulting tree based only on nucleotide substitutions) that are of the same size and in the same position in two unrelated sequences. In one case, both insertions represent direct repeats of a nine-bp adjacent sequence; in the other case, the six-bp insertions are not apparently related to adjacent sequences and share no nucleotides in common. The remaining two indels represent apparent synapomorphies. In one, a three-bp deletion is shared by *Digitalis*, *Veronica*, and *Plantago*, which together form a clade based on analysis of both of the gene sequences. In the other, a six-bp insertion forms an apparent synapomorphy for the entire Lamiales s.l., but gaps of the same size occur in the same position as the insertion in two ingroup sequences (*Nicodemia*, *Ajuga*) that are neither related to each other nor basal in the clade. Thus the 25 alignment gaps appear to represent 29 insertion or deletion events, of which two represent apparent synapomorphies of taxa in the current analysis. The phylogenetic significance of indels may be limited in this analysis, but their potential for phylogenetic inference at lower levels is great (see Discussion).

The parsimony analysis of *rbc*L sequences yielded eight equally most parsimonious trees (length = 851, consistency index, CI, = 0.426, excluding autapomorphies) occurring in two islands of six and two trees, respectively. The representatives of the Scrophulariaceae form two distinct clades (Figs. 2–4), designated "scroph I" (containing the type genus, *Scrophularia*, as well as *Celsia*, *Verbascum*, *Selago*, *Buddleja*, and *Nicodemia*) and "scroph II" (*Antirrhinum*, *Digitalis*, *Veronica*, *Plantago*, *Callitriche*, and *Hippuris*) on Figure 4. *Schlegelia* forms a clade with *Myoporum* in all *rbc*L trees and *Paulownia* forms a branch sister to the Bignoniaceae in the island of two trees or by itself near the base of the order in the island of six trees. Each island exhibits considerable resolution (trees not shown), but the strict consensus of all eight trees exhibits much less resolution (Fig. 2). The primary difference between the islands is in the placement of the Labiatae, which occur as sister group to a clade comprising the Acanthaceae and "scroph II" in island-2 (terminology of Maddison, 1991) and as a member of a large clade along with the Verbenaceae, Gesneriaceae, and "scroph I" in island-6. These results are congruent with those

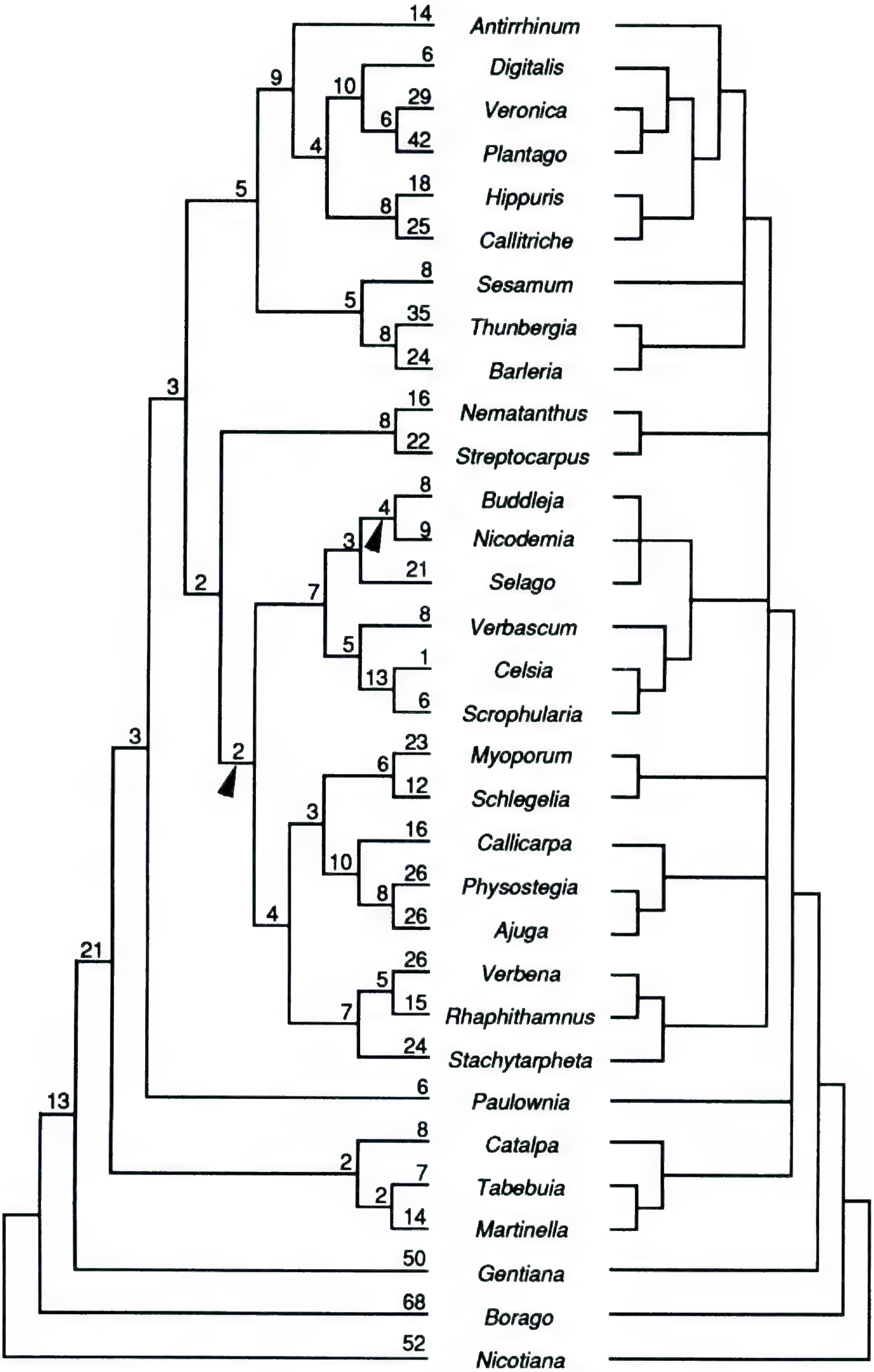

FIGURE 2. Results of parsimony analysis of *rbc*L sequences. On the left is one of six shortest trees in island-6 (length = 853) with branch lengths indicated. Arrows indicate internodes that collapse in the strict consensus of the six trees in island-6. On the right is a strict consensus of the eight shortest trees found, which includes the six trees in island-6 and the two trees in island-2.

obtained in previous analyses of *rbc*L sequences (e.g., Olmstead et al., 1993a).

The constrained search with a strict definition of Scrophulariaceae monophyly (*Antirrhinum, Celsia, Digitalis, Scrophularia, Selago, Verbascum, Veronica*) yielded 37 trees 24 steps longer than maximum parsimony (MP). A more relaxed constraint including *Plantago, Callitriche,* and *Hippuris* yielded 26 trees 9 steps longer than MP. Adding additional taxa, suggested by the maximum parsimony results to be potentially part of a monophyletic Scrophulariaceae clade, gave results as follows: (1) adding *Buddleja* and *Nicodemia,* 4 trees—3 steps; (2) adding *Buddleja, Nicodemia,* and *Myoporum,* 238 trees—8 steps. An additional analysis was performed, in which the *rbc*L sequences for *Byblis, Pinguicula,* and *Utricularia* (representing Lentibulariaceae) were retrieved from GenBank and added to the 32 sequences used elsewhere in this study. The results (not shown) indicate that the Lentibulariaceae represent a distinct lineage, not related to either of the two scroph clades identified in the analyses described above, confirming previously published results from a more limited sampling (Olmstead et al., 1993a).

The analysis of *ndh*F sequences yielded two trees (length = 2683, CI = 0.455, excluding autapomorphies). The representatives of the Scrophulariaceae form the same two distinct groups in the strict consensus tree (Fig. 3) as were found in the analysis of *rbc*L sequences. The closest groups to "scroph I" in order of increasing distance are Myoporaceae, Bignoniaceae, Acanthaceae, Verbenaceae, and Labiatae. "Scroph II" is sister group to the group just described. *Schlegelia* forms a clade with the Verbenaceae and *Paulownia* is sister group to the Labiatae. The constrained search with a strict definition of Scrophulariaceae yielded 4 trees 74 steps longer than MP. The more relaxed constraint (including *Plantago, Callitriche,* and *Hippuris*) yielded 10 trees 23 steps longer than MP. Adding additional taxa gave results as follows: (1) adding *Buddleja* and *Nicodemia,* 8 trees—8 steps; (2) adding *Buddleja, Nicodemia,* and *Myoporum,* 18 trees—4 steps.

The analysis of the combined *rbc*L and *ndh*F sequences yielded two trees (length = 3555, CI = 0.445 excluding autapomorphies). The same two distinct clades of Scrophulariaceae were obtained, and the branching order in the strict consensus tree (Fig. 4) is identical with respect to family-level groups to the results of the *ndh*F sequences. Results of the bootstrap analysis are shown in Figure 4. The constrained search with a strict definition of Scrophulariaceae yielded 3 trees 93 steps longer than MP. The more relaxed constraint (including *Plantago, Callitriche,* and *Hippuris*) yielded 5 trees 27 steps longer than MP. Adding additional taxa gave results as follows: (1) adding *Buddleja* and *Nicodemia,* 5 trees—9 steps; (2) adding *Buddleja, Nicodemia,* and *Myoporum,* 19 trees—4 steps. A decay analysis including all trees up to four steps longer than MP was performed to examine the degree of resolution remaining in the data, if a broadly defined Scrophulariaceae were to be accepted. In the strict consensus of the 1107 trees obtained, both "scroph I" and "scroph II" remain, as well as clades representing all of the families for which more than one representative was included (Buddlejaceae—within "scroph I," Acanthaceae, Bignoniaceae, Gesneriaceae, Labiatae, Verbenaceae) and the Lamiales s.l. as a whole. No other resolution within the ingroup is evident.

Discussion

Improved methods for obtaining DNA sequences have resulted in many systematists turning to direct sequence comparisons for molecular phylogenetic studies, rather than the indirect assessment of DNA sequence variation obtained using restriction site analysis. The initial widespread use of *rbc*L sequences was a product of two important factors: (1) the size and variability of the gene are appropriate for many systematic problems at the level of family and above and (2) primers for PCR amplification and sequencing were readily available to the plant systematics community. However, many of the problems of interest to plant systematists are at a level of phylogenetic divergence below which *rbc*L can provide sufficient information, or else represent problems of resolution among closely spaced branch points for which having more cladistic characters is desirable. Efforts to respond to these needs have resulted in the development of several alternative genes or regions of DNA for molecular phylogenetics (e.g., Baldwin, 1992; Johnson & Soltis, 1995, this issue; Olmstead & Sweere, 1994; Steele & Vilgalys, 1994), most of which have average substitution rates that are higher than that for *rbc*L (see Hoot et al., 1995, this issue, for an exception). For many studies at the infra- or interfamilial level, *rbc*L may still play a useful role in combination with another gene to increase the number of characters available (Olmstead & Sweere, 1994; Hoot et al., 1995, this issue; Johnson & Soltis, 1995; Soltis et al., 1993). In this study, the numbers of informative characters obtained from two genes, *rbc*L and *ndh*F, expressed in terms of characters per base pair of

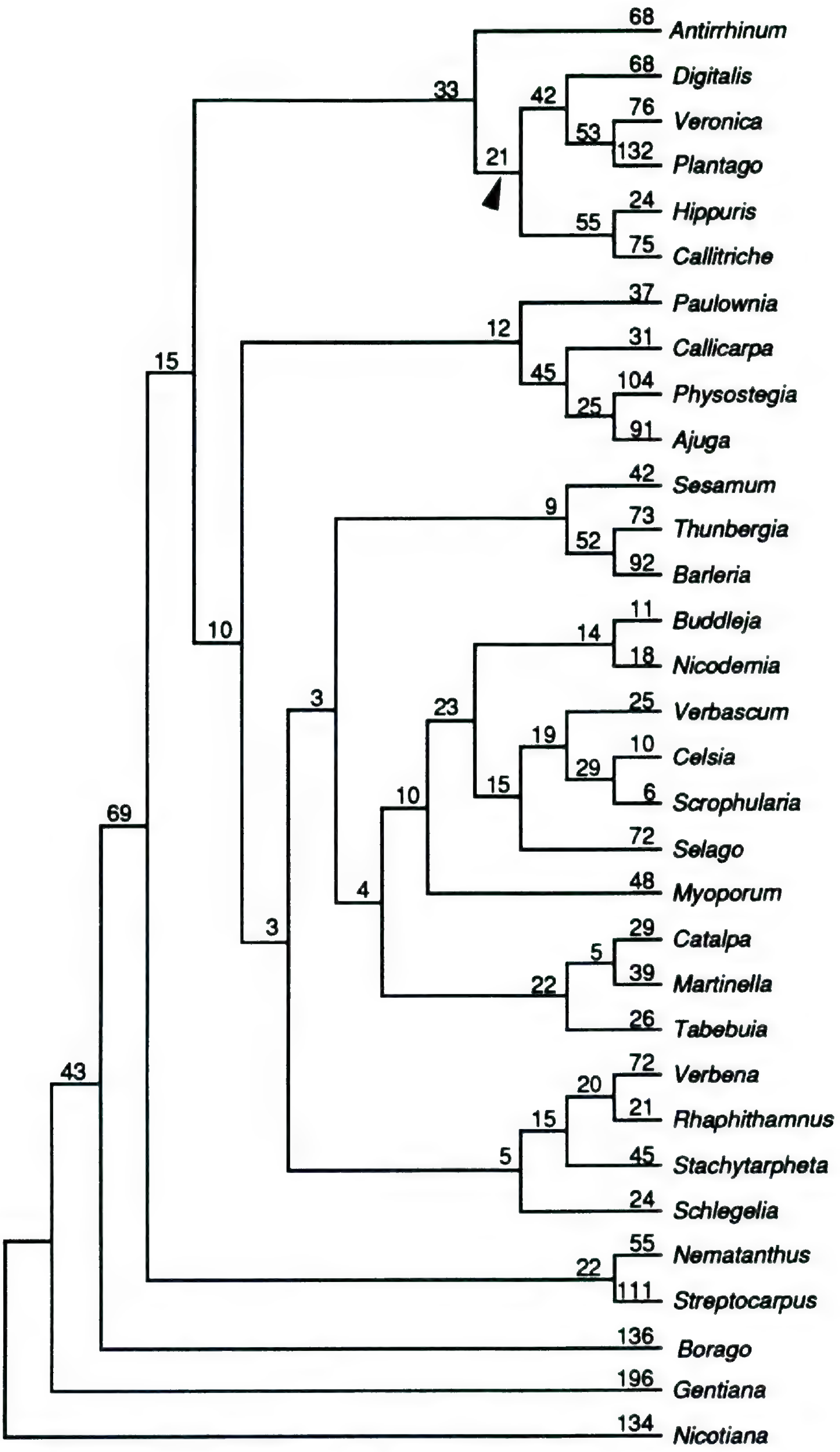

FIGURE 3. One of the two shortest trees resulting from parsimony analysis of *ndh*F sequences (length = 2683) with branch lengths indicated. Arrow indicates internode that collapses in strict consensus of the two trees.

useful aligned sequence, are 0.13 and 0.26 respectively, and the amount of sequence obtained was 50% greater for *ndh*F than for *rbc*L. Thus *ndh*F yielded three times as many characters as did *rbc*L. The problem of lineage identification (e.g., family-level clades) in the Lamiales s.l. and the relationship among them has been identified as one for which more data than are available from *rbc*L may be needed for resolution (Olmstead et al., 1993a).

*ndh*F sequences afford more than simply an additional source of nucleotide substitutions for phylogenetic inference. Indels are a regular, if infrequent, occurrence in the middle of the coding sequence of *ndh*F. This is unlike *rbc*L, for which insertions and deletions are unknown, except at the very end of the coding sequence where it is often difficult to discriminate insertions from point mutations in the termination codon resulting in an extension of the 3′ end. This feature of the gene may yield powerful phylogenetic characters that may be useful in many studies of infrafamilial phylogeny as has been demonstrated for *mat*K (Johnson & Soltis, 1995).

The alignment of protein-coding sequences containing indels is simplified, relative to noncoding DNA or rRNA coding genes, by the constraint of the genetic code for protein-coding genes. Alignments "by eye" are easier by virtue of including three nucleotides in the alignment frame. A group of relatively closely related species is unlikely to be so divergent that alignment matches will be obscured. In contrast, in noncoding or rRNA sequences indels may be of any length, and the problem of aligning one-base or two-base gaps is much greater. In the event that divergence of protein coding DNA sequences is great enough that alignment "by eye" is difficult, aligning the translated amino acid sequences may often resolve the placement of gaps that are not apparent in the DNA sequences.

The 25 alignment gaps in the set of *ndh*F sequences used in this study represent 29 evolutionary events as compared to the 2683 nucleotide substitution events (as inferred from the results of the cladistic analysis of the nucleotide information). The overall consistency index for nucleotide substitutions (including autapomorphies) is 0.56, whereas the same estimate for indels is 0.86. In this analysis, most of the indels were uninformative; however, each of the autapomorphic indels may be informative at a lower taxonomic level, as was found in several instances with *mat*K in the Saxifragaceae s.s. and Polemoniaceae (Johnson & Soltis, 1995). In the Solanaceae (Olmstead & Sweere, 1994), two additional indels were documented, each of which was unique to one of the 17 species included in that study. However, the nine-bp indel unique to *Nicotiana* in this study is common to all members of the Solanaceae examined and found in none of the other sequences yet obtained in the Asteridae, including those for the sister family Convolvulaceae, thereby providing a synapomorphy for the Solanaceae. In the Acanthaceae (Scotland et al., 1995), 14 indels were identified, of which 8 were unique to a single sequence and 6 were phylogenetically informative. Of the six informative ones, only one was an evident case of parallelism, and one other required two events in the shortest tree but could be explained as a single event in a tree two steps longer.

The observation of parallel occurrence of these infrequent events is more understandable when one takes into consideration the nonrandom distribution of indels in the sequence (Table 2, Fig. 5). Nineteen gaps, representing 21 inferred events, occur in a 148-bp region (between positions 1425 and 1573 in *Nicotiana*), and another 4 gaps (6 events) occur in an 18-bp region (1695–1713 in *Nicotiana*). Only two gaps, each representing a unique event, occur outside these regions (insertions at position 657/658 and 1932/1933). The higher incidence of indels is associated with higher substitution rates in the 3′ half of the *ndh*F sequence (Olmstead & Sweere, 1994), making that portion of the gene, by itself, appropriate for some phylogenetic studies (Catalan & Olmstead, unpublished).

The results of three separate analyses are presented (Figs. 2–4): *rbc*L sequences, *ndh*F sequences, and a combined data set with both sequences. The case has been made for conducting separate analyses for data sets derived from different genes (e.g., Swofford, 1991) in the event that one sequence exhibits strong interactions among positions that may bias substitutions, thereby violating the assumption of nonindependence among characters. However, no evidence of such differences has been found in prior analyses of *rbc*L and *ndh*F sequences (Olmstead & Sweere, 1994; Scotland et al., 1995), and none is anticipated here. Performing the separate analysis does afford the opportunity to examine the efficacy of each gene for phylogeny reconstruction and to examine the effect of partitioning a data set to see whether strongly supported clades in one partition are equally supported in another (Olmstead & Sweere, 1994). The results of the analyses of the two individual data sets for this group of taxa (Figs. 2–3) are congruent with respect to finding the same clades that are the most well-supported clades in the combined analysis (Fig.

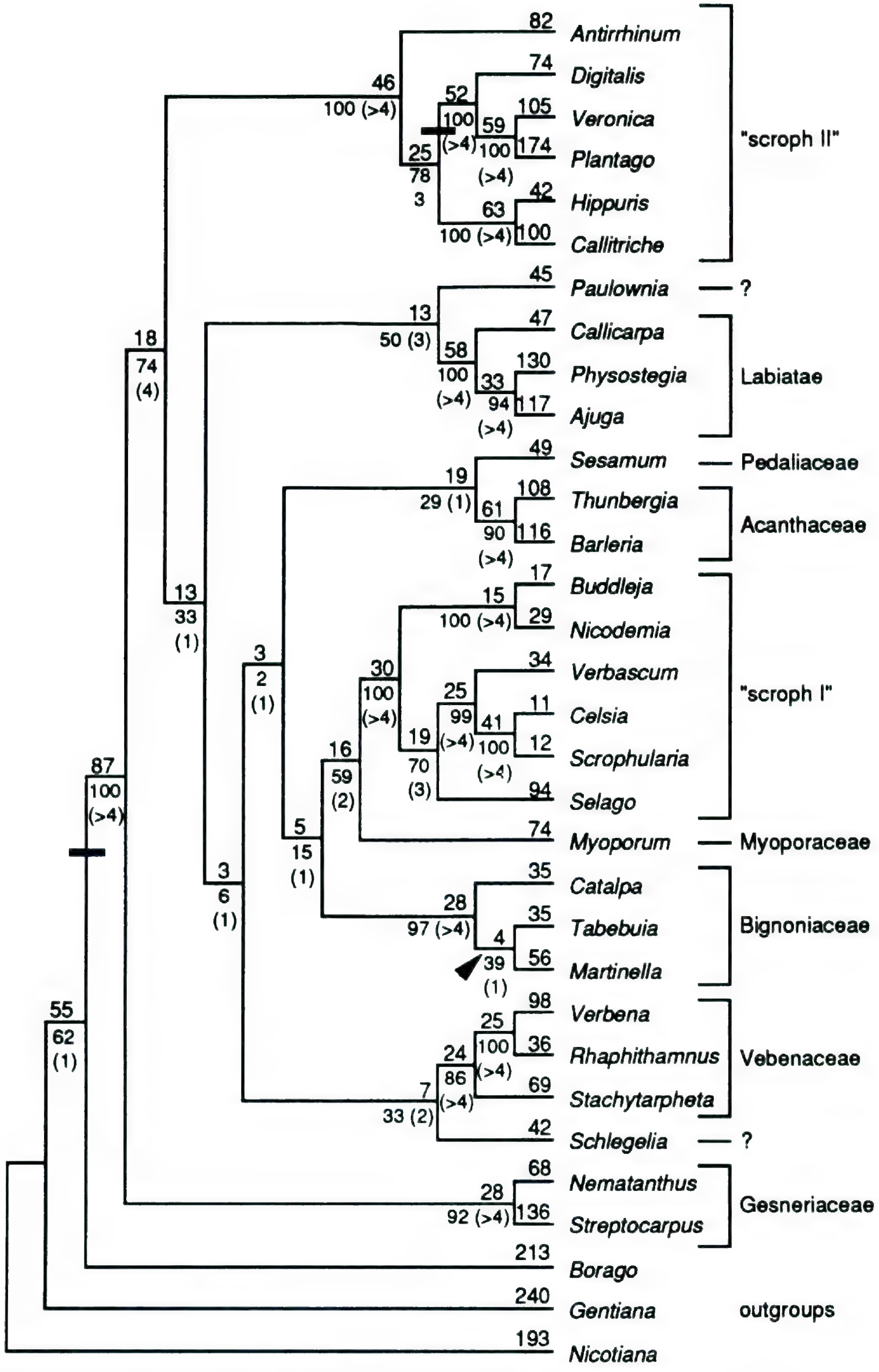

FIGURE 4. One of the two shortest trees resulting from parsimony analysis of *rbc*L and *ndh*F sequences combined (length = 3555) with branch lengths indicated above the internodes (the other tree is the same as Fig. 3). Numbers below the internodes indicate bootstrap and decay (in parentheses) values. Arrow indicates internode that collapses

4). The differences between the two results occur in areas where support is weak in both individual trees. Groups identified by *rbc*L, but not *ndh*F, all had five or fewer synapomorphies on the *rbc*L tree, and groups identified by *ndh*F, but not *rbc*L, all had 12 or fewer synapomorphies on the *ndh*F tree; groups in common in both results had much higher character support. The results of the combined analysis (Fig. 4) are nearly identical to the results of the *ndh*F analysis (Fig. 3), with one of the two most parsimonious trees found in each analysis being identical. However, *rbc*L informs the combined result in several ways: (1) *ndh*F does not fully resolve internal relationships in the "scroph II" clade, whereas *rbc*L and the combined analyses do. (2) *ndh*F and *rbc*L resolve the relationships among the three representatives of the Bignoniaceae differently; the combined analysis remains unresolved. (3) Most importantly, at almost every node where the combined tree agrees with the *ndh*F result, but not the *rbc*L result, *rbc*L provides additional characters in support of the conclusions, indicating that phylogenetic signal is present in the "*rbc*L partition" for some portions of the tree where very few characters are informative and may be outweighed by chance homoplasy in that relatively small partition of the data.

SYSTEMATIC CONCLUSIONS

The primary systematic conclusion of this study is that the Scrophulariaceae, as circumscribed in most taxonomic treatments, are polyphyletic. The polyphyly of the Scrophulariaceae is complex in the following ways: (1) There exist two distinct clades in this study (and possibly more in nature) that contain elements of the traditional Scrophulariaceae (referred to as "scroph I" and "scroph II" in Fig. 4). (2) There are groups that are excluded from the family in traditional classifications that are clearly related to and derived from elements of the traditional Scrophulariaceae (e.g., Plantaginaceae, Callitrichaceae, Hippuridaceae, and apparently Orobanchaceae, as noted previously, which is not included in this study). (3) Some disputed genera, assigned to the Scrophulariaceae in some treatments (e.g., *Schlegelia* and *Paulownia* in Thorne, 1992) do not belong to either "scroph I" or "scroph II."

The results of cladistic analysis of data sets derived from both *rbc*L and *ndh*F identify most of the same terminal lineages within the Lamiales s.l. Represented in these lineages are the Acanthaceae, Labiatae, Verbenaceae, Bignoniaceae, Gesneriaceae, and two groups of taxa containing elements of the traditional Scrophulariaceae. The "scroph I" lineage consists, in this study, primarily of elements that are distinctive and often isolated within or near the traditional Scrophulariaceae: *Verbascum* and *Celsia* (Antirrhinoideae, Verbasceae), *Selago* (Antirrhinoideae, Selagineae), *Buddleja* and *Nicodemia* (Buddlejaceae), which are often placed close to the Scrophulariaceae, but not within it (e.g., Cronquist, 1981), and the type genus *Scrophularia* (Antirrhinoideae, Scrophularieae). The Verbasceae, which have nearly actinomorphic flowers, were previously classified as subfamily Pseudosolaneae (Wettstein, 1895) which suggested connections to the Solanaceae. In fact, Bentham and Hooker (Bentham, 1876) placed the solanaceous tribe Salpiglossideae, which have zygomorphic flowers, unlike most Solanaceae, in the Scrophulariaceae in recognition of the similarity. The Selagineae have been segregated from the Scrophulariaceae (e.g., Bentham & Hooker in Bentham, 1876) into their own family primarily on the basis of the reduction in ovule number to one per carpel (Thieret, 1967). Reduction in ovule number to one or two per carpel, at times with each carpel subdivided into two uniovulate mericarps, has occurred independently in the Lamiales s.l. on several occasions (Wagstaff & Olmstead, unpublished). The Buddlejaceae are provisionally included in the "scroph I" clade, rather than as sister group to it (as suggested by the *ndh*F and combined results), because the *rbc*L results include them within the otherwise completely scroph group and the bootstrap and decay values determined for the combined data set do not unequivocally reject that hypothesis. Recognition of the fact that the genus *Scrophularia* belongs in this group means that a phylogenetic classification would have to recognize "scroph I" or some portion of it as Scrophulariaceae, and "scroph II" would require a new family name.

The "scroph II" lineage consists of the traditional scrophs *Antirrhinum* (Antirrhinoideae, Antirrhineae), *Digitalis* (Rhinanthoideae, Digitaleae),

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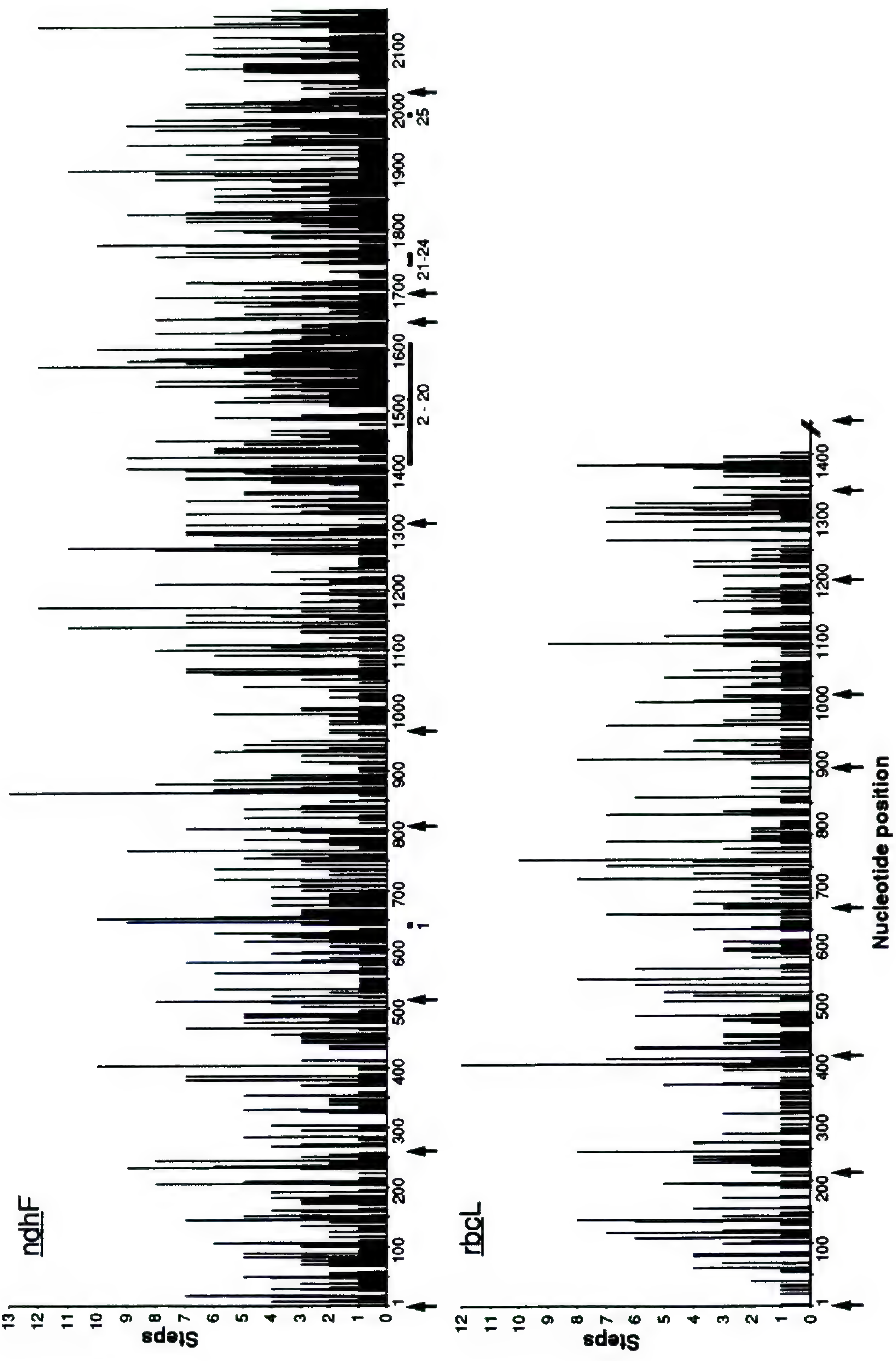
in strict consensus of the two trees. Dark bars indicate a deletion (within "scroph II") and an insertion (at the base of the Lamiales s.l.) in the *ndh*F sequences. Question marks (?) next to *Paulownia* and *Schlegelia* indicate that their classification into a higher order taxon remains uncertain.


ndhF
rbcL
Steps
Nucleotide position
2 - 20
21-24
25
1

and *Veronica* (Rhinanthoideae, Veroniceae), and representatives of the derived families Callitrichaceae, Hippuridaceae, and Plantaginaceae. Thieret (1967) suggested that the Scrophulariaceae be divided into two subfamilies with the Antirrhinoideae ancestral to the Rhinanthoideae. In this analysis, members of the Antirrhinoideae are included in both "scroph I" and "scroph II" lineages, making the subfamily polyphyletic, whereas the sampled members of the Rhinanthoideae form a monophyletic group with *Plantago*. The Rhinanthoideae contain all of the hemiparasitic Scrophulariaceae, from which the Orobanchaceae are evidently derived (C. dePamphilis, pers. comm.; A. Colwell, pers. comm.), thereby suggesting that *Orobanche* and relatives belong in this clade. The Plantaginaceae exhibit modifications to floral morphology associated with wind pollination, which have resulted in their segregation as a separate family, while retaining enough similarity to be recognized as close to or derived from the Scrophulariaceae. However, the aquatic families Callitrichaceae and Hippuridaceae exhibit extremely modified reproductive morphology, which has baffled previous efforts to classify them with their closest relatives in the Scrophulariaceae. The third aquatic family in Cronquist's (1981) Callitrichales, the Hydrostachyaceae, has been found to be unrelated to the Callitrichaceae and Hippuridaceae and is most closely related to the Hydrangeaceae in the Cornales on the basis of *rbc*L sequences (Hempel et al., in press).

The phylogenetic position and classification of *Schlegelia* and *Paulownia* remain unclear. *rbc*L and *ndh*F sequences each suggest a different placement for *Schlegelia* (near Myoporaceae—*rbc*L; near Verbenaceae—*ndh*F) and *Paulownia* (isolated near the base of the order or with the Bignoniaceae—*rbc*L; near Labiatae—*ndh*F). The combined analysis provides relatively weak evidence for the placement of *Schlegelia* near the Verbenaceae (33% bootstrap value) and *Paulownia* near the Labiatae (50% bootstrap value). The evidence does suggest that neither genus belongs with either of the lineages containing other members of the Scrophulariaceae. *Schlegelia*, and the related genera *Gibsoniothamnus* and *Synapsis*, may represent one of several small lineages in the Lamiales s.l. that have been "shoehorned" into families representing larger lineages in the past. The Lentibulariaceae appear to represent another small independent lineage (Olmstead et al., 1993a) that has been suggested incorrectly to be a derivative of the Scrophulariaceae (e.g., Cronquist, 1981). An expanded analysis of *rbc*L sequences, including three representatives of the Lentibulariaceae in addition to the 32 taxa in this study, indicates that the Lentibulariaceae are not related to either of the scroph lineages identified here (results not shown).

Given that the phylogenetic hypothesis derived from the parsimony analysis of the cpDNA sequences presented here may not be the true phylogeny of the group, it is instructive to examine prospective circumscriptions of groups that may represent a monophyletic Scrophulariaceae. This was done by imposing on the parsimony analysis constraints that require a group to be monophyletic, while finding the most parsimonious trees. Several prospective circumscriptions were examined, beginning with a very strict definition matching most recent classifications and expanding to a broad circumscription that includes all taxa belonging to the lineages "scroph I" and "scroph II" and *Myoporum*. Not surprisingly, parsimony analyses constrained to the narrowest circumscriptions resulted in trees that were much longer than maximum parsimony (e.g., 93 steps longer for the most conservative definition of Scrophulariaceae in the combined analysis of *rbc*L and *ndh*F sequences). However, a broadly circumscribed Scrophulariaceae, including the Plantaginaceae, Callitrichaceae, Hippuridaceae, Buddlejaceae, and *Myoporum* are monophyletic in trees four steps longer than maximum parsimony in the combined analysis.

If the results of a parsimony analysis were to be used as evidence for classification in which a group that requires trees four steps longer than maximum parsimony might be acceptable, then it is relevant to ask how many trees are found and how much resolution is retained when all trees up

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FIGURE 5. Histograms representing the changes by nucleotide position inferred over the shortest estimated tree for the combined data (tree in Fig. 4) for the genes *ndh*F and *rbc*L. The nucleotide position numbers refer to the aligned sequences used in the phylogenetic analysis beginning with the first nucleotide after the 5′ PCR primer and ending with the last nucleotide before the 3′ PCR primer. Arrows indicate locations of the PCR and sequencing primers. The bars under the *ndh*F sequence indicate the locations of gaps in the sequences (numbers refer to Table 2).

to four steps longer than the shortest are examined. A decay analysis saving all trees up to four steps longer than maximum parsimony found 1107 trees. The consensus among those trees shows that all families for which more than one representative were included are supported and that both "scroph I" and "scroph II" lineages are supported, but no other resolution is possible within the Lamiales s.l. Whereas there is no assurance that the shortest tree is the true phylogeny for this group of taxa, the fact that the "scroph I" and "scroph II" lineages are obtained even in the consensus of the trees up to four steps longer suggests that their classification as two distinct groups comparable to other groups resolved at this level (i.e., family) would be a conservative move and would produce a more stable classification when further evidence is obtained.

The debate has subsided, but no consensus has been reached on the distinction between polyphyly and paraphyly (or even monophyly—see Cronquist, 1987). Even Hennig used different criteria for the terms, first defining polyphyly on the basis of shared, convergent characters and paraphyly on the basis of shared, ancestral characters (Hennig, 1966), then (Hennig, 1975) distinguishing the two by whether the common ancestral (stem) species of a group would be included (paraphyly) or not included (polyphyly) in that group. The point at which the debate impacts systematics and classification most strongly centers on whether a greater distinction needs to be made between monophyletic versus para- and polyphyletic groups (Donoghue & Cantino, 1988) or mono- and paraphyletic versus polyphyletic groups (Cronquist, 1987). This study serves a useful purpose by illuminating how difficult and pointless it is to try to distinguish between paraphyly and polyphyly as opposed to monophyly. Perhaps a group that is defined on the basis of plesiomorphic characters or by the inclusion of a stem species could be recognized and named Scrophulariaceae, but such a classification would serve poorly any evolutionary applications, because virtually all of the Lamiales s.l. could be included in an evolutionary lineage identified by those traits or derived from that stem species. The fact that progress is being made to identify and circumscribe monophyletic families successfully in the Lamiales s.l. argues against the continued recognition of a traditional Scrophulariaceae and for the recognition of family-level groups that were once part of the Scrophulariaceae. The circumscription and formal recognition of such groups awaits the completion of work in progress (C. dePamphilis, R. Olmstead & A. Wolfe, unpublished). It is hoped that the recognition of this division within the Scrophulariaceae based on cpDNA will encourage additional morphological and anatomical work aimed at identifying monophyletic groups containing elements of the traditional Scrophulariaceae.

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THE UTILITY OF *atp*B GENE SEQUENCES IN RESOLVING PHYLOGENETIC RELATIONSHIPS: COMPARISON WITH *rbc*L AND 18S RIBOSOMAL DNA SEQUENCES IN THE LARDIZABALACEAE[1]

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ABSTRACT

The chloroplast gene *atp*B was sequenced for seven genera of the Lardizabalaceae and three outgroup taxa to assess its utility as a source of phylogenetic information. The resulting phylogenetic tree was compared with trees based on 18S nuclear ribosomal DNA and *rbc*L (chloroplast DNA) sequences, as well as a combination of all data (*atp*B, 18S, and *rbc*L) for the same taxa. Sequence divergence values, statistics related to patterns of character transformation, and indices measuring homoplasy and branch support were also compared. The topology of the trees derived from *atp*B, 18S, and a combination of all three sequence data sets were largely congruent. All phylogenies, with the exception of the tree derived from *rbc*L data, supported the monophyly of the Lardizabalaceae. All indicators of nucleotide substitution rate suggest that *rbc*L is the least conserved, *atp*B is intermediate, and 18S is the most conserved of the three genes sequenced. Measures of homoplasy also indicate that the *rbc*L tree is less strongly supported than those based on *atp*B, 18S, or a combination of *atp*B, 18S, and *rbc*L sequence data.

Phylogenetic analyses of higher-level plant groups using DNA sequence data have been based most often on the chloroplast gene *rbc*L or, less frequently, on 18S nuclear ribosomal DNA (18S nrDNA). Few phylogenetic studies have used other gene sequences across a broad range of taxa, and still fewer have compared results from two or more gene sequences for the same taxa (e.g., Baldwin, 1992; Johnson & Soltis, 1994; Olmstead & Sweere, 1994). As part of an intensive systematic study of phylogenetic relationships among basal eudicots (Ranunculidae and "lower" Hamamelididae), we have further developed the chloroplast gene, *atp*B, as a new source of phylogenetically informative data (Ritland & Clegg, 1987). Here, we apply the *atp*B gene to resolve phylogenetic relationships in the angiosperm family Lardizabalaceae and compare the results with those based on *rbc*L and 18S nrDNA for the same taxa. Cladograms based on the three genes are evaluated and compared in terms of their resolution and congruence, as well as various measures of phylogenetic signal, transition/transversion bias, sequence divergence, and homoplasy. Based on these data, we discuss the effectiveness of using each gene for phylogenetic studies at the generic level and above.

The Lardizabalaceae (Ranunculidae, sensu Takhtajan, 1987—"ranunculids") are a family of twining (rarely erect) shrubs found in temperate areas of Eastern Asia and South America. The family is characterized by alternate, palmate (rarely pinnate) compound leaves; regular unisexual flowers; six overlapping or valvate sepals (three in *Akebia*); staminodia or petals small or absent; three

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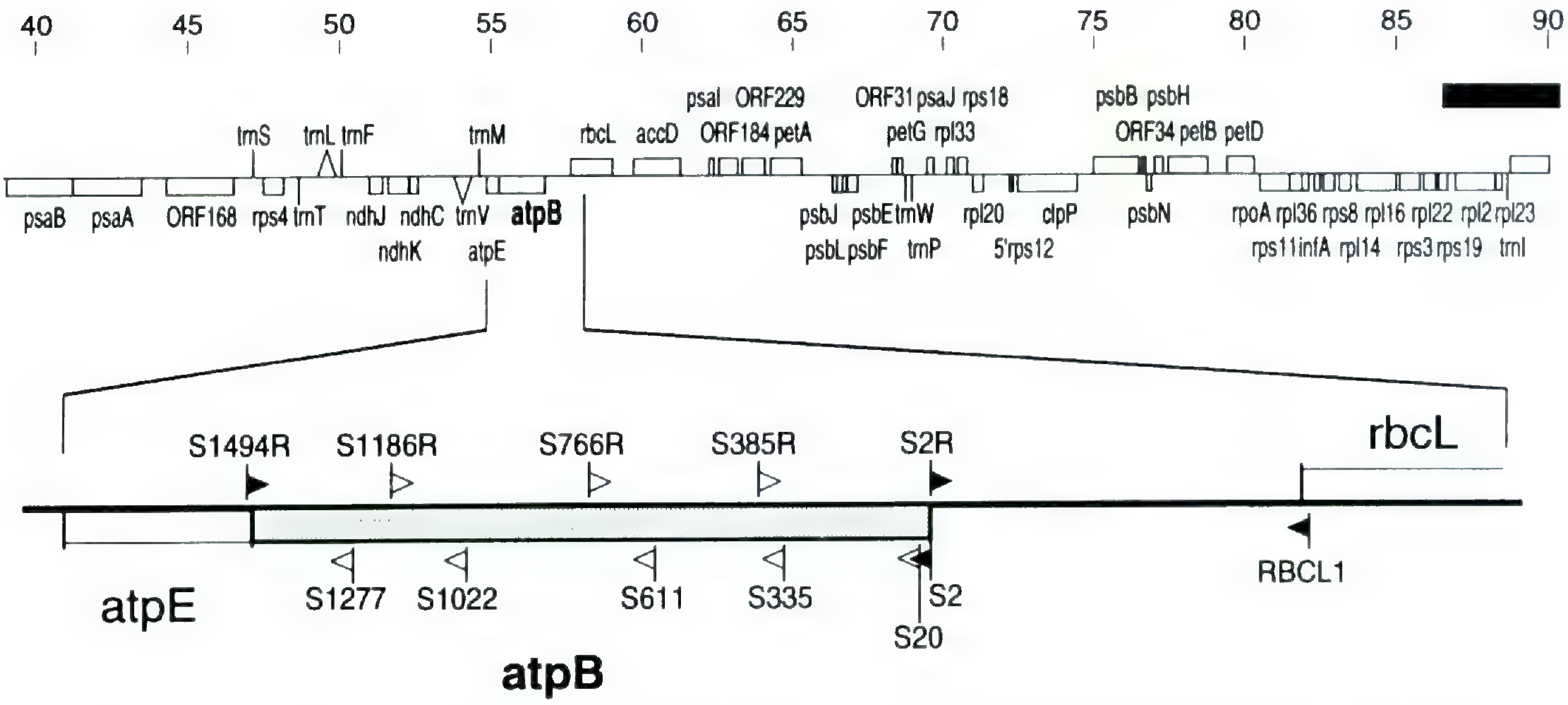


FIGURE 1. Location of the *atpB* gene in relation to a portion of the tobacco chloroplast genome. Top numbers indicate tobacco coordinate units × 1000 (TCU; Shinozaki et al., 1986). Line below the TCU scale maps a portion of the single copy region and inverted repeat of the tobacco chloroplast genome from TCU 40,000 to TCU 90,000. Black bar at approximately 87,000 TCUs is the beginning of the inverted repeat. Genes above the tobacco genome map (Shinozaki et al., 1986) are transcribed from left to right; those below the line are transcribed from right to left. The bold line beneath the tobacco genome map illustrates the bracketed region above in more detail. Filled in arrows indicate the location and direction of the amplification primers for the *atp*B gene. Hollow arrows indicate the location and direction of the internal sequencing primers.

carpels (up to nine in *Akebia*); numerous ovules (four, by abortion, in *Boquila*) with laminar placentation (submarginal in *Decaisnea* and *Sinofranchetia*).

Previous phylogenetic analyses based on *rbc*L sequence data suggest that the Lardizabalaceae occupy a key and potentially basal position in the evolution of the Ranunculidae, but the family was represented by only the genus *Akebia* (Chase et al., 1993). This molecular study was undertaken to clarify intergeneric relationships in the family, to test the results of previous morphologically based cladistic analyses (Loconte & Estes, 1989), and to provide a more secure basis for representing the family in our ongoing investigation of basal eudicot radiation. Of particular interest are the phylogenetic positions of *Decaisnea* and *Sinofranchetia* (the only Lardizabalaceae genera with sub-marginal placentation) with respect to the other genera and the relationships of the two dioecious South American genera, *Boquila* and *Lardizabala*, the only representatives of the family outside eastern Asia.

THE *atp*B GENE

The *atp*B gene is located in the large single-copy region of the chloroplast genome contiguous with the *atp*E gene and downstream from the *rbc*L gene, from which it is separated by an approximately 900 bp intergenic spacer region (Fig. 1). The *atp*B gene encodes the β subunit of ATP synthase (other subunits are encoded in either the chloroplast or the nuclear genomes). ATP synthase has a highly conserved structure that couples proton translocation across membranes with the synthesis of ATP (Zurawski et al., 1982; Gatenby et al., 1989). Previous to this study, the chloroplast *atp*B gene had been sequenced for approximately ten genera, representing a diverse range of plants (e.g., *Chlamydomonas*, *Marchantia*, *Spinacia*, *Nicotiana*, and *Oryza*).

Many features of the *atp*B gene suggest that it may be valuable for comparative sequence studies at higher taxonomic levels. It is short enough (1497 bp) for ease of sequencing but long enough to be potentially phylogenetically informative, given broadly comparable rates of evolution to *rbc*L. The evolutionary rate is conserved and K_s (a measure of the rate of synonymous nucleotide substitution in the gene; Li et al., 1985; Wolfe, 1991) between rice and tobacco is 0.62, indicating a rate of evolution very similar to that found for *rbc*L (K_s = 0.63; Wolfe, 1991). There are no reported insertions and deletions in the *atp*B gene, the gene does not contain introns, and *atp*B sequences are readily aligned.

MATERIALS AND METHODS

DNA EXTRACTION AND AMPLIFICATION

The seven genera of Lardizabalaceae and three outgroup genera used in this study are indicated

TABLE 1. Species sequenced, bases sequenced for each gene (only missing data of ≥10 nucleotides are noted as gaps), total number of variable sites missing from each sequence (listed in parentheses), sources of sequences (SH—Sara Hoot, AC—Alastair Culham), and voucher information with notations on nature of material (fresh, silica-dried, or herbarium). Accession numbers (GSDB) are in the following order: *atp*B, 18S, and *rbc*L.

Species	*atp*B Bases sequenced and source	18S Bases sequenced and source	*rbc*L Bases sequenced and source	Accession information and location of voucher
Decaisnea fargesii Franchet	25–358, 390–1493 (5) SH	39–1710 (1) SH	31–1261, 1276–1428 (3) SH	L37926, L37907, L37916 Reznicek 9236, MICH (fresh)
Lardizabala biternata Ruíz & Pavón	57–1493 (0) SH/AC	39–1710 (0) SH	31–1428 (0) SH/AC	L37929, L37910, L37919 Edinburgh 271032, F (fresh)
Holboellia latifolia Wallich		39–1710 (1) SH		L37909 Culham 193, RNG (silica-dried)
Holboellia latifolia Wallich	57–1493 (2) SH/AC		31–1428 (1) SH/AC	L37928, L37918 Edinburgh 715977, F (fresh)
Sinofranchetia chinensis Hemsley	61–1493 (1) SH/AC	39–1710 (3) SH	31–1300 (15) SH	L37931, L37912, L37921 Edinburgh 831635, F (fresh)
Stauntonia hexaphylla (Thunb.) Decne.	25–1493 (0) SH	39–1411, 1433–1710 (2) SH	31–1428 (1) SH	L37932, L37913, L37922 Lammers 8465, F (silica-dried)

TABLE 1. Continued.

Species	*atp*B Bases sequenced and source	18S Bases sequenced and source	*rbc*L Bases sequenced and source	Accession information and location of voucher
Akebia quinata Decne.			29–1428 (1) Qiu	L12627 Qiu 91020, NCU
Akebia quinata Decne.	68–364, 388–1493 (5) SH	39–1710 (0) SH	31–300 SH	L37924, L37905 Hoot 925, F (fresh)
Boquila trifoliata Decne.	19–368, 381–1493 (2) SH/AC	39–595, 615–1710 (1) SH	31–1428 (5) SH/AC	L37925, L37906, L37915 Gardner & Knees 4088, RNG (1988) (Herbarium specimen)
Dicentra eximia Torrey	25–379, 401–1493 (4) SH	39–1170, 1191–1710 (2) SH	34–1428 (3) SH	L37927, L37908, L37917 Reznicek 9756, MICH (fresh)
Nandina domestica Thunb.	60–339, 388–1493 (7) SH	39–1710 (1) SH	67–780, 813–1428 (8) SH	L37930, L37911, L37920 Matthaei Bot. Gard., Hoot 922 F (fresh)
Tinospora caffra Miers	59–1493 (1) SH	39–1710 (0) SH	31–1315, 1329–1421 (1) SH	L37933, L37914, L37923 Jaarsveld 2131, NBG (silica-dried)

TABLE 2. Location (position based on *atp*B sequence for *Spinacia*, Zurawski et al., 1982) and base composition of amplification and internal sequencing primers for the chloroplast gene, *atp*B. All primers were designed by S. Hoot.

	Amplification primers
RBCL1	5′ GAA TCC AAC ACT TGC TTT AGT CTC T (for amplification of the spacer region between *atp*B and *rbc*L as well as the *atp*B gene)
S2	5′ TAT GAG AAT CAA TCC TAC TAC TTC T (amplifies the *atp*B gene only)
S1494R	3′ TCA GTA CAC AAA GAT TTA AGG TCA T
	Internal sequencing primers, forward direction
S20	5′ CTT CTG ATC CTG GGG TTT CCA CAC T
S335	5′ ACG TGC TTG GGG AGC CTG TTG ATA A
S611	5′ AAC GTA CTC GTG AAG GAA ATG ATC T
S1022	5′ CGA CAT TTG CAC ATT TAG ATG CTA C
S1277	5′ AAA TTG AGC GTT TCT TAT CAC AAC C
	Internal sequencing primers, reverse direction
S2R	5′ AGA AGT AGT AGG ATT GAT TCT CAT A
S385R	5′ GCG CAG ATC TAT GAA TAG GAG ACG T
S766R	5′ TAA CAT CTC GGA AAT ATT CCG CCA T
S1186R	5′ TGT CCT GAA GTT CTT TGT AAC GTT G
S1494R	same as amplification primer above

in Table 1, along with sequencing, accession, and voucher information. Total cellular DNA was isolated from fresh, silica-dried, or herbarium material according to the miniprep method of Doyle & Doyle (1987). In some cases (*Boquila trifoliata*, *Sinofranchetia chinensis*, *Stauntonia hexaphylla*), DNA was further purified and concentrated after extraction using GeneClean (GeneClean, Bio 101, Inc.).

A segment of double-stranded DNA containing most of the coding sequence (approximately 1474 bp) for the *atp*B gene was amplified using the polymerase chain reaction (PCR). Three amplification primers were designed using *atp*B sequences available from GenBank for spinach, tobacco, pea, sweet potato, maize, and wheat. Two alternate 25-mer 5′ primers were used. One is located at the 5′ end of the *rbc*L coding sequence (nucleotide positions 15–39 in tobacco). It has the opposite orientation from *rbc*L but the same orientation as *atp*B, and includes the intergenic spacer region between *rbc*L and *atp*B in the amplification product (Table 2, Fig. 1). The other primer begins with the first base upstream of the *atp*B start codon and includes the first 24 bases of the *atp*B coding sequence (positions 1–24 in spinach). The 3′ 25-mer amplification primer, S1494R, is located at the 5′ end of the adjacent gene, *atp*E (positions 1–25 of the *atp*E gene in spinach), and has the opposite orientation as the *atp*B gene (Table 2).

Two alternative protocols differing only in $MgCl_2$ concentration and annealing temperature were used to amplify *atp*B. In the first protocol, the reaction mixture contained (either final concentrations or amounts in a 100 μl reaction): 10 mM Tris-HCl, pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 0.5 μM of each amplification primer, 2.5U Taq Polymerase, 0.3–2.0 μL template DNA (depending on concentration). To prevent evaporation during thermal cycling, a drop of mineral oil was added to each reaction mixture. The sample was then placed in a thermocycler (M. J. Research, Inc., Cambridge, Massachusetts) with the following cycling parameters: premelt at 92°C for 3 min.; 30 cycles, each consisting of a denaturation step at 92°C for 1 min., annealing step at 55°C for 1 min., and an extension step at 72°C for 3 min.; followed by a final extension step of 72°C for 7 min. The alternative protocol included the following modifications: the concentration of $MgCl_2$ was doubled and the annealing step temperature lowered to 50°C. In most cases one of these two protocols produced amplification product. In those cases where yield was still very weak, further amplification directly from the Gene-Cleaned amplification product often produced increased yields.

In most cases, the amplification primers for the chloroplast gene *rbc*L were those described in Olmstead et al. (1992). In *Sinofranchetia*, no amplification product could be obtained using these primers. Substituting a 3′ primer located at position

1300 in the tobacco *rbc*L sequence and using an 18S amplification protocol (Nickrent, 1993) resulted in high yields (Table 1). 18S nrDNA was amplified by using either the primers and protocols of Nickrent (Nickrent, 1993; Nickrent & Starr, 1993) or those of Hamby et al. (1988).

Particular attention was given to purification of PCR products to avoid superimposed sequences that can result from priming by the amplification primers as well as the internal sequencing primer during double-standed sequencing. Samples were run on a 2% low melt agarose gel (NuSieve GTG) with 1% TAE buffer and ethidium bromide, bands were visualized by means of UV illumination, then removed as gel plugs. To remove agarose and concentrate the PCR product, gel plugs were melted at 65°C for approximately 10 min., then further purified and concentrated with glass milk (GeneClean). This procedure proved especially important to obtain high-quality sequences for *rbc*L and 18S nrDNA but amplification products for *atp*B frequently gave excellent results when sequenced after using only the GeneClean purification process.

DOUBLE-STRANDED SEQUENCING

The purified dsPCR product was sequenced directly with the dideoxy-termination method and Sequenase T7 DNA polymerase (US Biochemical) using the protocol of Thien (1990) with the following modifications: the addition of 1% acetamide to the annealing reaction and an incubation temperature of 46–47°C for the termination step. Internal sequencing primers for *atp*B are shown in Table 2 and Figure 1. Sequencing of *rbc*L used a combination of internal primers, kindly provided by G. Zurawski (DNAX Research Institute, Palo Alto, California) and a few primers designed specifically for this study (positions and sequences available from SBH). The 18S internal primers used to date were generously furnished by D. Nickrent (Nickrent & Starr, 1993) or E. A. Zimmer (Hamby et al., 1988).

Aliquots of the sequencing reactions were loaded on two 60 × 33 cm field-gradient 6% polyacrylamide gels and subjected to electrophoresis overnight (short run—600V, long run—1100V). After transferring to 3MM Whatman paper, the gels were vacuum-dried for approximately one hour at 80°C and exposed to autoradiography film for 1–3 days. Typical autoradiographs from gels run in this manner yielded 300 to 350 readable bases. All sequences used in this study are available from GSDB or directly from the senior author. Consistently, *atp*B proved the easiest to sequence of the three genes examined: it amplified readily, suffered the least from multiple banding patterns when the PCR product was not gel-purified, and often yielded readable sequences 300–350 bp from the internal primers.

QUALITY CONTROL OF SEQUENCE DATA

Sequence comparisons for the genes *atp*B, 18S, and *rbc*L included 1468, 1671, and 1397 bp, respectively (Table 1). Both strands of DNA were sequenced for both *atp*B and *rbc*L with approximately 80% overlap. Both strands were also sequenced for 18S, but with much less overlap between the two directions (30–40%). The sequences were read from the autoradiographs, recorded on a data sheet, entered into MacClade (Maddison & Maddison, 1992), then printed and rechecked from the autoradiographs for errors.

Alignment problems (caused by compressions) for *atp*B and *rbc*L often could be rectified by reading the opposite strand. Within the *atp*B gene, the following regions were susceptible to compressions: positions 47–53, 875–879, and 1455–1457. There were several regions in the 18S nrDNA sequences where alignment was impossible because of compressions or base insertion/deletion events. These regions were deleted from the data matrix and are located at the following positions in relation to the soybean 18S sequence (Eckenrode et al., 1985): 224–231, 667–670, 710, 738, 1174–1175, 1366, and the very end of the amplified region, 1711–1761. Sequence divergence values (described below) calculated between pairs of sequences excluded these problematic regions or positions. The possibility of PCR-generated anomalous sequences was checked by comparison of sequences from closely related taxa. Sequences furnished by other labs were also checked for inconsistencies both by comparison with other closely related taxa and occasional duplicate sampling of the same genus (e.g., the *rbc*L and 18S sequences for *Akebia*, and the *rbc*L sequence for *Dicentra*).

DATA ANALYSIS

Phylogenetic analyses were performed using PAUP 3.1 (Swofford, 1993) using the branch-and-bound search option (with collapse of zero-length branches) to assure recovery of the most parsimonious trees. PAUP was also used to perform bootstrap analysis with 1000 replications using the branch-and-bound search option (Felsenstein, 1985). The decay indices (the number of steps that must be added to the minimal-length tree before a

TABLE 3. Comparison of data sets from *atp*B, 18S nrDNA, *rbc*L, and a combination of all data. Numbers in parentheses indicate number of informative three-state characters (excluding those where two of the three states were autapomorphies). The % Ts = unambiguous transitions/unambiguous changes × 100 and was calculated for each gene from one of the most parsimonious trees. g_1 is a measure of the skewness of the distribution of 100,000 randomly generated trees. Tree length (TL) was calculated including uninformative characters; consistency index (CI) and rescaled consistency index (RC) were calculated excluding uninformative characters. RI = retention index.

Gene	Variable sites	Informative sites	Binary characters	3-state characters	4-state characters	%Ts	g_1	Number of trees	TL	CI	RI	RC
*atp*B	143	42	36	11 (6)	0	69	−1.36	3	172	0.73	0.74	0.54
18S	76	25	21	6 (4)	0	66	−1.19	3	88	0.81	0.85	0.69
*rbc*L	172	56	47	21 (9)	0	56	−0.59	1	225	0.64	0.68	0.44
Combined data	391	123	104	38 (19)	0	61	−0.82	2	493	0.67	0.70	0.47

clade collapses) were computed for all trees using the heuristic search option (Donoghue et al., 1992). A tree length distribution of 100,000 randomly sampled trees was generated for each of the *atp*B, 18S, and *rbc*L data matrices using the "random trees" selection of PAUP. This distribution was analyzed for skewness as an estimate of nonrandom structure in the sequences (Hillis & Huelsenbeck, 1992). Sequence divergence values were computed as the proportion of divergent sites from direct pairwise comparisons of the sequence data.

There has been much discussion about the advantages and disadvantages of analyzing independent data sets separately, combining independent data sets prior to phylogenetic analyses, or analyzing them separately and then applying consensus methods (see Bull et al., 1993, for a review). There is clearly a strong argument for combining data sets, especially in cases where there is substantial homoplasy and the phylogenetic signal in a particular data set is insufficient to resolve certain branching patterns in a tree (Kluge, 1989; Barrett et al., 1991; Olmstead & Sweere, 1994). For this reason, we have chosen to analyze a combination of all the data sets (*atp*B, 18S, and *rbc*L) as well as each data set separately.

Alternative tree topologies and resultant changes in tree length were explored using MacClade 3.0 (Maddison & Maddison, 1992). MacClade was also used to calculate character transformations of various types for each sequence tree (for example, transition/transversion bias and the number of changes at different codon positions).

Outgroup taxa for the Lardizabalaceae in all analyses were selected based on the results of several previous phylogenetic analyses of the Ranunculidae (sensu Takhtajan, 1987). A cladistic analysis based on traditional data placed Berberidaceae, Menispermaceae, and the Ranunculineae (Ranunculaceae plus Papaveraceae) as potential sister taxa to the Lardizabalaceae (Loconte & Stevenson, 1991). However, preliminary analyses of *atp*B and *rbc*L data (analyzed as separate and combined data sets) with extensive sampling of the ranunculids, consistently place the Ranunculaceae as the most derived family of the Ranunculidae. Representatives from two families, Menispermaceae and Berberidaceae, are the basal members of a large clade that is resolved as the sister group to the Lardizabalaceae (Hoot & Crane, work in progress). The Papaverales are resolved as relatively basal to the Lardizabalaceae and other ranunculids (Chase et al., 1993; Hoot & Crane, work in progress). Therefore, in this paper *Dicentra eximia* (Fumariaceae, Papaverales) was used to root the phylogenetic analyses with *Tinospora* (Menispermaceae) and *Nandina* (Berberidaceae) included as additional outgroup taxa (Table 1).

RESULTS

PHYLOGENETIC ANALYSES

Table 3 lists the number of variable positions, informative characters (after removal of autapomorphies), and binary, three- and four-state characters for each gene.

Analysis based on the *atp*B data resulted in three equally parsimonious trees; *Stauntonia, Akebia,* and *Holboellia* are unresolved due to lack of variable sites. These trees were based on 143 variable sites (Table 3; 42 informative characters) with a tree length (TL) = 172, a consistency index excluding autapomorphies (CI) = 0.73 (Kluge & Farris, 1969), and a retention index (RI) = 0.74

(Farris, 1989). The strict consensus cladogram derived from the three trees is presented in Figure 2. The monophyly of the Lardizabalaceae is well supported, with 19 base substitutions, a bootstrap value of 100%, and a decay index of 11. *Sinofranchetia* and *Decaisnea* are basal within the family. The remaining five genera form two clades, each of which is also supported by morphological characters (Hoot, Culham & Crane, work in progress). The clade consisting of the two South American genera, *Boquila* and *Lardizabala*, is only weakly supported (one base substitution, a bootstrap value of 64%, and a decay index of one) in contrast to stronger support for the clade comprising the Asian genera, *Stauntonia*, *Akebia*, and *Holboellia* (four base substitutions, a bootstrap value of 94%, and a decay index of three).

The 18S nrDNA data matrix, consisting of 76 variable sites (25 informative sites), resulted in three most parsimonious trees with a TL = 88, CI (excluding autapomorphies) = 0.81, and RI = 0.85. One of the shortest trees is presented in Figure 2 (the branch that collapses in the strict consensus tree is indicated with dotted lines). As in the *atp*B tree, the monophyly of the Lardizabalaceae with respect to the three outgroups is well supported with nine nucleotide changes, a bootstrap value of 99%, and a decay index of seven. The 18S tree is congruent with the *atp*B tree in other respects as well: *Sinofranchetia* and *Decaisnea* are basal within the family and the clade consisting of *Lardizabala*, *Boquila*, *Akebia*, *Stauntonia*, and *Holboellia* is recognized but with less internal resolution.

The *rbc*L data consisting of 172 variable sites (56 informative sites) resulted in one fully resolved most parsimonious tree (Fig. 2). The TL = 225, CI (excluding autapomorphies) = 0.64, and RI = 0.68. However, unlike the results from *atp*B and 18S, the *rbc*L sequence data do not support the monophyly of the Lardizabalaceae, placing *Sinofranchetia* with the outgroup *Dicentra* in a basal position with respect to all other genera. The *rbc*L tree is congruent with the *atp*B tree in placing *Decaisnea* as the sister genus to the clade consisting of *Lardizabala*, *Boquila*, *Akebia*, *Stauntonia*, and *Holboellia*, although the pattern of relationships among these five genera is different (the clade containing *Lardizabala* and *Boquila* is not recognized).

A further analysis performed using a combination of all three data sets (*atp*B, *rbc*L, and 18S nrDNA) resulted in two trees derived from 391 variable sites (123 informative sites) with a TL = 493, CI (excluding autapomorphies) = 0.67, and RI = 0.70. The clade that collapses in a strict consensus tree of the two most parsimonious trees is shown with dotted lines (Fig. 2). Excluding relationships among outgroups, the consensus tree based on all data is most similar to the tree based on *atp*B data alone. The monophyly of the Lardizabalaceae is again strongly supported with 37 nucleotide changes, a bootstrap value of 100%, and a decay index of 14. The combined data also support the clades consisting of (*Lardizabala*, *Boquila*, (*Akebia*, *Stauntonia*, and *Holboellia*,)) but with higher bootstrap values and decay indices (Fig. 2).

CHARACTER TRANSFORMATIONS

The number of substitutions inferred for each nucleotide position was calculated over the trees derived from the three sequence data sets using MacClade 3.0 (Maddison & Maddison, 1992). For both *atp*B and *rbc*L, substitutions occur fairly uniformly across the gene (Fig. 3). Even considering the exclusion of some nucleotide positions due to compressions or insertion/deletion events (see above), the nucleotide substitutions are much less evenly distributed across the 18S gene. This is probably due to constraints imposed by the secondary structure of 18S nrDNA (Gutell & Woese, 1990; Nickrent & Sargent, 1991; Dixon & Hillis, 1993). There is variation in the number of steps/site found for each gene, with a high of four steps in *atp*B to seven in *rbc*L.

Most of the unambiguous changes (142 of 172 changes or 83%) in the most parsimonious *atp*B trees occur at third-position sites contrasted with 15 unambiguous changes each at first and second positions. A similar pattern is found with the *rbc*L tree: 41 changes in the first-position, 21 in second-position, and 167 in third-position sites (73%). The CIs (including autapomorphies) for the various positions are high for both genes, even at third-position sites: 1.0 (*atp*B) and 0.73 (*rbc*L) for first positions, 0.93 and 0.90 for second positions, and 0.88 and 0.86 for third positions.

There are approximately twice as many transitions (94–95) as transversions (42–43) when calculated over the three most parsimonious *atp*B trees. A similar proportion of transitions to transversions occurs with the 18S data, 51–52 transitions to 25–26 transversions. However, the proportion is more even when calculated over the most parsimonious *rbc*L tree, 64 transitions to 51 transversions.

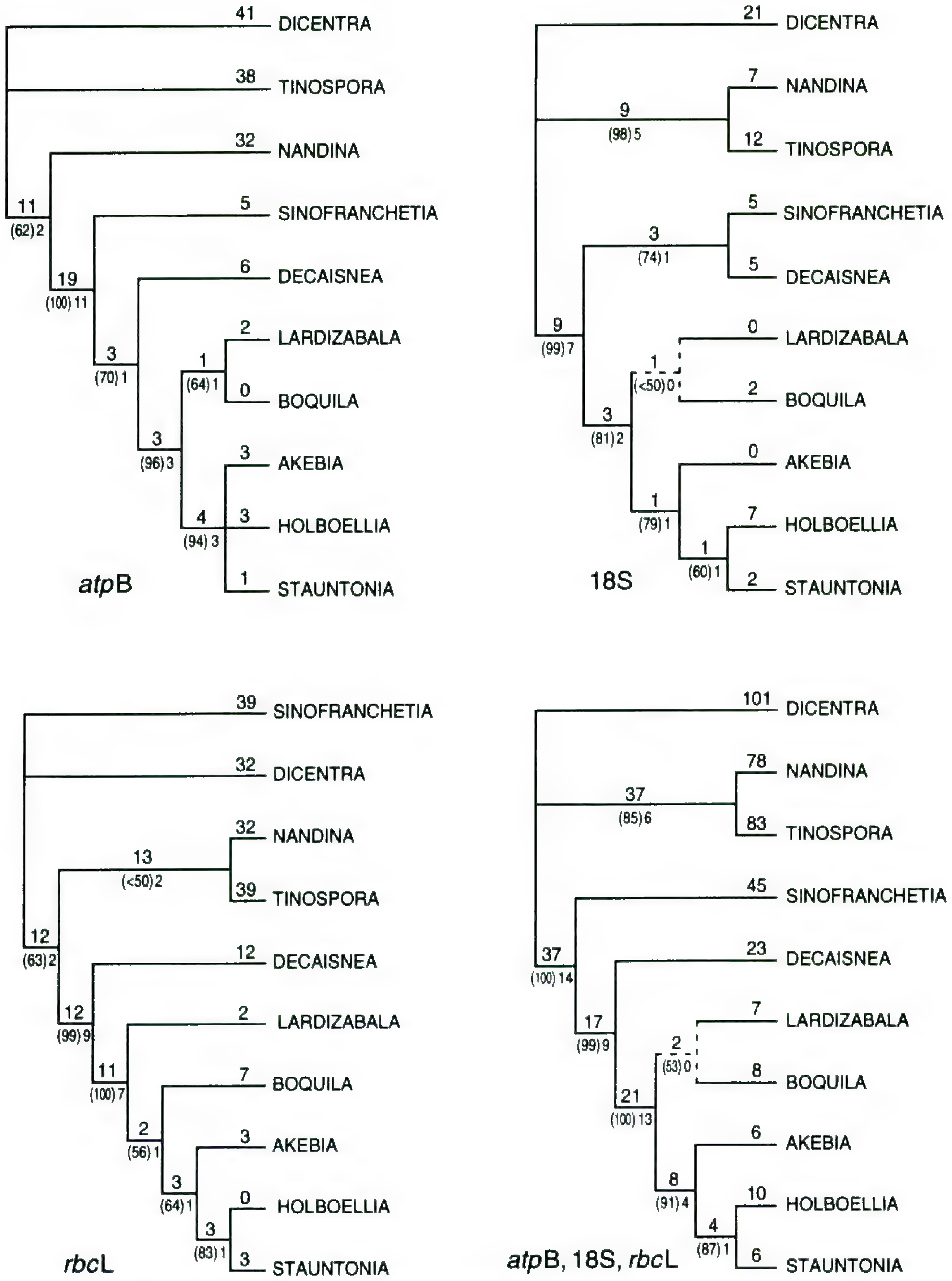
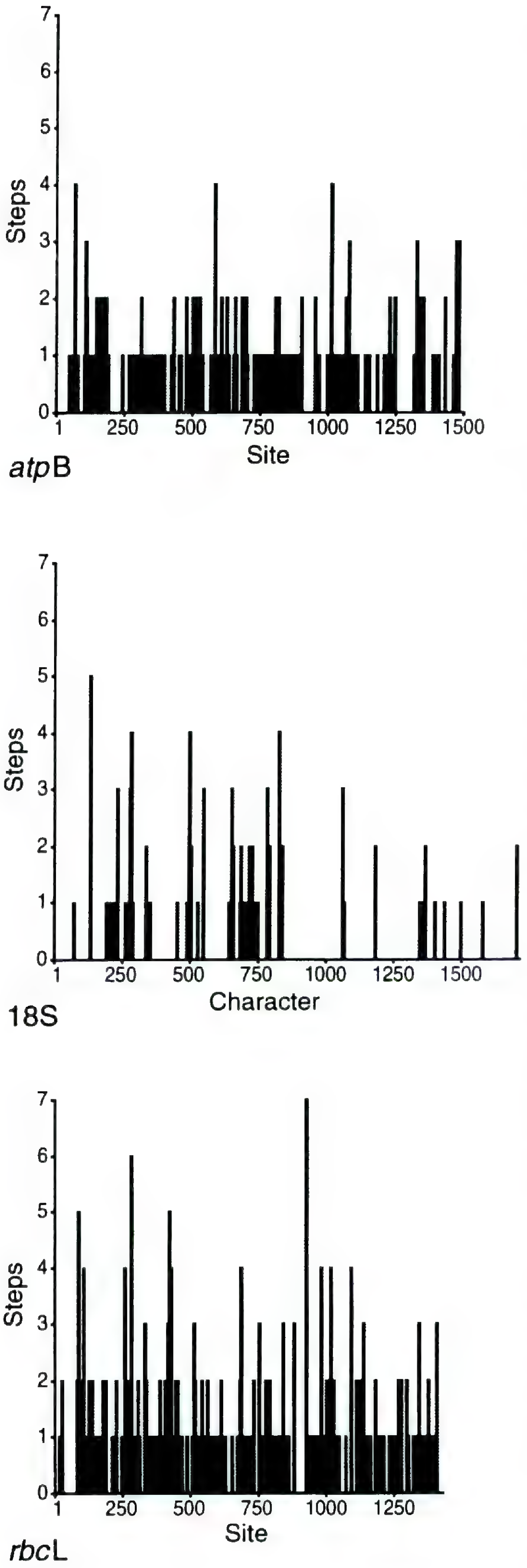


FIGURE 2. Most parsimonious phylogenetic trees resulting from *atp*B, 18S nrDNA, *rbc*L, and a combination of all three sequence data sets. Numerals above branches indicate the number of nucleotide changes supporting each branch. Numerals below in parentheses indicate the percentage of times that the branch was recovered in 1000 bootstrap replications. Numbers below and to the right of the bootstrap values are decay indices, indicating how many additional steps are necessary before the branch collapses. For 18S and a combination of all three data sets, only one of several equally parsimonious topologies (with appropriate values) is illustrated. Dotted lines in the trees based on 18S and a combination of all three data sets indicate where branches collapse in the strict consensus trees derived from multiple most parsimonious trees.



COMPARISON OF SEQUENCE DIVERGENCE AND PHYLOGENETIC SIGNAL

Table 4 presents the sequence divergence values for *atp*B, 18S, and *rbc*L gene sequences from selected pairs of genera chosen to cover the entire range of divergence found within this study. The highest divergence values are found in pairwise comparisons with the outgroup genus *Dicentra*. Comparing divergence between the three genes, the highest values are found in the *rbc*L sequences (5.2%), followed by *atp*B (4.8%) and 18S (2.5%). The range narrows in pairwise comparisons between more closely related genera, with no substantial difference in the values found in the pairwise comparison of *Stauntonia* and *Holboellia* for the three genes (0.2–0.6%). Divergence values were calculated simply as a proportion of divergent sites in each sequence comparison with no provision made to account for superimposed events (multiple hits) which must have occurred at many positions. Using this algorithm, once an initial substitution has occurred at a position, subsequent changes at that same position cannot increase the divergence, but divergence can be decreased by converting the novel nucleotide back to the original condition (parallelism or reversal). For this reason, divergence values do not increase uniformly with the number of substitution events, but instead increase rapidly at first and more slowly thereafter (Swofford & Olsen, 1990). A graph of sequence divergence values resulting from pairwise comparisons of *Stauntonia* with four genera is shown in Figure 4. While the divergence values for *atp*B and 18S increase with more distantly related taxa, there is a noticeable flattening of the curve with *rbc*L (Fig. 4, Table 4). This suggests that, at greater taxonomic distances, superimposed events caused by more frequent substitutions are more of a factor in this *rbc*L data set and that some of the estimates of sequence divergence for this gene are probably artificially low.

The distribution of the lengths of 100,000 randomly generated trees for the *atp*B sequence data reflects considerable nonrandom structure (Fig. 5). The skewness of this distribution, measured by a g_1 value of -1.36, far exceeds the $P = 0.01$ critical value for data sets of this size (critical values of g_1 for four-state characters, 10 taxa, and 100/250

←

FIGURE 3. Histograms showing distribution of nucleotide changes and the number of steps/site or character as calculated from the most parsimonious phylogenetic trees derived from *atp*B, 18S nrDNA, and *rbc*L sequence data.

TABLE 4. Pairwise divergence computed between representative genera for *atp*B, 18S nrDNA, and *rbc*L gene sequences. Divergence values in the upper right half of each matrix are the proportion of divergent sites in each comparison. Actual number of divergent sites calculated from the original data matrix appears in the lower left half of each matrix. Sequences were not compared at positions with missing or ambiguous states.

*atp*B	1	2	3	4	5
1. Dicentra	0	0.044	0.045	0.048	0.046
2. Sinofranchetia	62	0	0.010	0.012	0.011
3. Lardizabala	64	14	0	0.006	0.005
4. Holboellia	68	17	9	0	0.003
5. Stauntonia	67	15	7	4	0
18S rDNA	1	2	3	4	5
1. Dicentra	0	0.022	0.020	0.025	0.022
2. Sinofranchetia	35	0	0.007	0.012	0.009
3. Lardizabala	32	12	0	0.007	0.004
4. Holboellia	41	20	12	0	0.006
5. Stauntonia	36	14	7	9	0
*rbc*L	1	2	3	4	5
1. Dicentra	0	0.052	0.046	0.047	0.049
2. Sinofranchetia	67	0	0.046	0.042	0.043
3. Lardizabala	64	60	0	0.006	0.008
4. Holboellia	65	54	9	0	0.002
5. Stauntonia	68	56	12	3	0

variable characters were −0.33 and −0.27 respectively, Hillis & Huelsenbeck, 1992). The g_1 values for 18S nrDNA and *rbc*L (−1.19 and −0.59, Table 3) were lower but also significant.

DISCUSSION

The trees derived from all three genes and the combined data give broadly similar phylogenetic results. All analyses support the recognition of a clade comprising *Akebia*, *Stauntonia*, and *Holboellia* within a broader clade that includes *Lardizabala* and *Boquila*. An important morphological feature that is diagnostic of this inclusive clade is laminar placentation. The two Lardizabalaceae with submarginal placentation (*Decaisnea* and *Sinofranchetia*) are placed external to this group. The relatively basal position of *Decaisnea* and *Sinofranchetia* is consistent with the results of prior phylogenetic analyses based on traditional data and classification schemes (Loconte & Estes, 1989; Qin, 1989). There is also weak support (from *atp*B, 18S, and combined data) for the two South American taxa (*Boquila* and *Lardizabala*) as sister genera.

The efficacy of the *atp*B gene for phylogenetic reconstruction is well supported by the number of variable sites and the relatively high consistency and retention indices in our *atp*B analysis (Table 3). However, another measure of the accuracy of the emergent phylogenies is provided by comparing the *atp*B tree with trees derived from other data sets. The *atp*B tree is largely congruent with the trees derived from 18S nrDNA and with a tree derived from a combination of all three data matrices (Fig. 2). However, unlike the trees resulting from *atp*B and 18S nrDNA alone, and a combination of all the molecular data, the *rbc*L tree does not recognize the monophyly of the Lardizabalaceae nor does it recognize the *Boquila* and *Lardizabala* clade. All possible pairwise combinations of the *atp*B, 18S, and *rbc*L data sets also support the monophyly of the Lardizabalaceae, although only the combination of *atp*B and 18S gave a single tree that supported the sister relationship of *Boquila* and *Lardizabala*. Results from the *rbc*L data are therefore somewhat incongruent with those from other genes, various combined data sets, and also morphological studies. Since Decaisne's work on the family (1837–1838, 1839), the Lardizabalaceae have been considered a natural family (Prantl, 1891; Taylor, 1967; Hutchinson, 1973; Cronquist, 1981; Takhtajan, 1987; Qin, 1989), and this is supported by a previous cladistic analysis of the Ranunculales (Loconte & Estes, 1989), as well as preliminary analyses of the family based on morphology (Hoot, Culham & Crane, work in progress). There are two unambiguous characters within

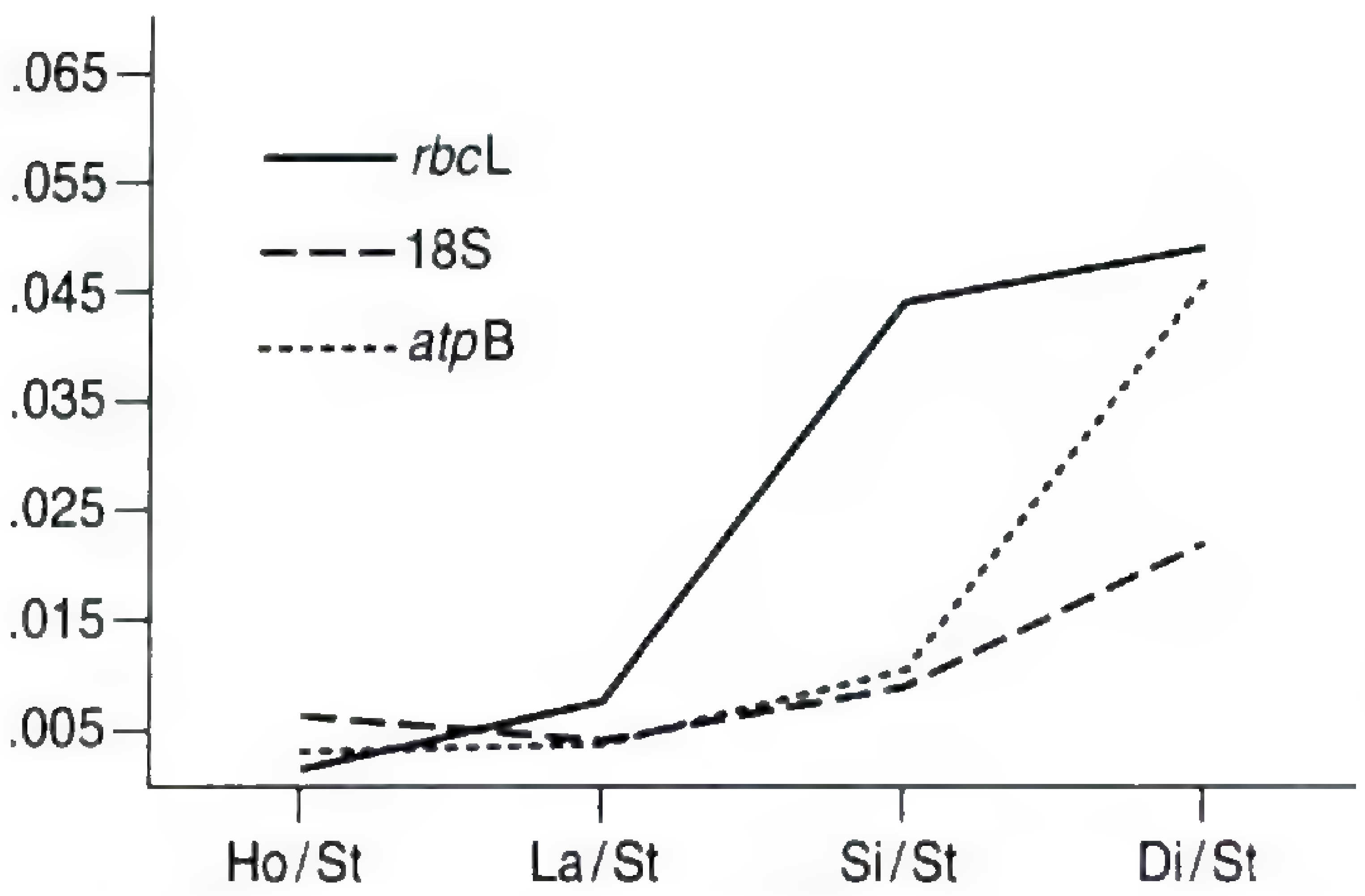


FIGURE 4. Graph of divergence values in pairwise comparisons of selected genera with *Stauntonia*. Ho = *Holboellia*, St = *Stauntonia*, La = *Lardizabala*, Si = *Sinofranchetia*, and Di = *Dicentra*.

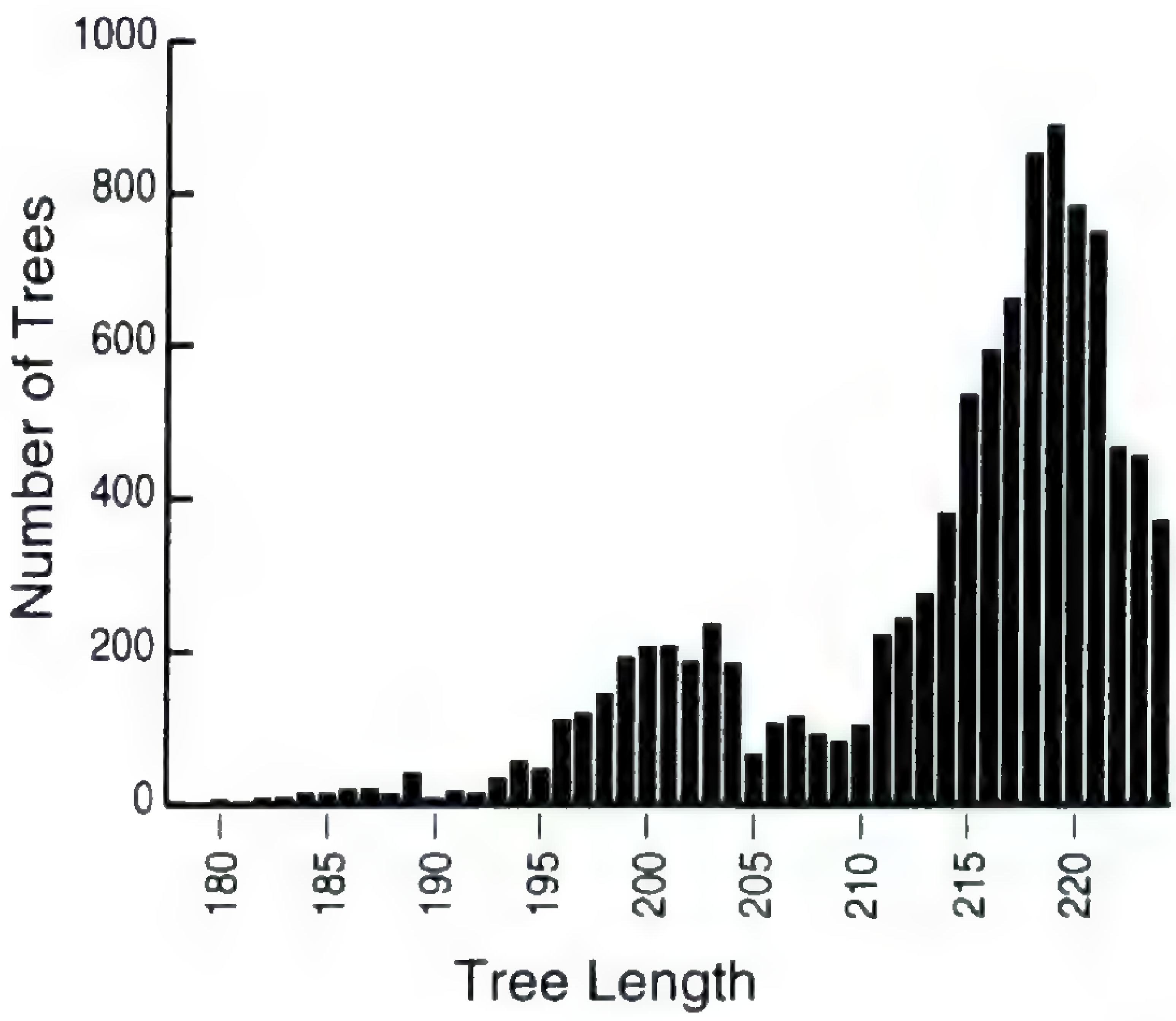


FIGURE 5. Histogram showing skewness ($g_1 = -1.36$) of tree lengths for 100,000 randomly generated trees derived from *atp*B sequence data.

the *rbc*L data set that support the monophyly of the Lardizabalaceae but they are outweighed by eight unambiguous characters supporting the inclusion of *Sinofranchetia* with the outgroup, *Dicentra*. Combining the *rbc*L data set with either the *atp*B or the 18S rDNA data set is sufficient to counteract the *rbc*L characters supporting polyphyly, resulting in trees recognizing a monophyletic Lardizabalaceae.

The statistics and indices of support for particular tree topologies and branching patterns also favor the monophyletic status of the Lardizabalaceae, as opposed to the separation of *Sinofranchetia* favored by the *rbc*L tree. Table 3 presents the various CIs, RIs, and rescaled consistency indices (RC; Farris, 1989). By any of these measures, trees supporting the family's monophyly exhibit less homoplasy than is found with the *rbc*L tree. The number of nucleotide changes, the bootstrap values (99–100%), and decay indices (7–14) for the branch supporting the monophyly of the family are extremely high in the trees with this topology. Furthermore, moving *Sinofranchetia* basal to all the rest of the Lardizabalaceae as found in the *atp*B, 18S, and combined-data trees adds only four steps (1.7% of the tree length excluding noninformative characters) to the *rbc*L tree length. In contrast, despite the smaller number of informative sites, moving *Sinofranchetia* to any outgroup position costs a minimum of 12 steps (6.9% of TL) in the *atp*B tree, seven steps (7.9% of TL) in the 18S tree, and 15 steps in the combined data tree (3.0% of TL).

Several experiments were conducted on the *rbc*L data set to test how firmly *Sinofranchetia* was separated from other genera of Lardizabalaceae. There were two potentially informative characters (at tobacco positions 1345 and 1380) missing from the *Sinofranchetia rbc*L sequence due to amplification problems (see Materials and Methods). To test whether these missing data were responsible for the reduced support for Lardizabalaceae monophyly, we inserted the character states found in other Lardizabalaceae. This produced a single most parsimonious tree with exactly the same topology as the *rbc*L tree (Fig. 2). Assuming that positions with more than two states may be particularly subject to mutation, a further analysis was performed in which all positions with three or more character states were omitted from the *rbc*L data set. This also resulted in a tree with the same topology as was found with the full *rbc*L data set.

The lack of support for Lardizabalaceae monophyly may be, at least in part, a "long-branch" attraction effect between *Dicentra* and *Sinofranchetia*, caused by the relatively higher rate of substitutions in *rbc*L compared with the other two sequences. The higher number of variable characters, the larger number of positions with three-state characters, less left-handed skewness in the data set ($g_1 = -0.59$; Table 3), and the higher sequence divergence values between matched pairs of taxa (Table 4) indicate a higher rate of nucleotide substitutions. Removal of third-position sites results in the exclusion of both *Decaisnea* and *Sinofranchetia* from the Lardizabalaceae (a similar experiment with the *atp*B data yields a consensus tree less resolved but congruent with that found in Fig. 2). This suggests that substitution rates within the *rbc*L gene are at a level where saturation is just as likely to occur at some first and second positions as third positions. The homoplasy values calculated

for first-, second-, and third-position sites (0.27, 0.10, and 0.14, respectively) in this and other phylogenetic studies using *rbc*L sequences support this conclusion (Donoghue et al., 1992; Kim et al., 1992; Chase et al., 1993).

All possible configurations of outgroup taxa (i.e., only *Nandina, Tinospora*, or *Dicentra* included, or each removed, leaving two outgroup representatives) were tested for their effect on the resolution of the *rbc*L data. Only the removal of *Dicentra* resulted in a monophyletic Lardizabalaceae. Frequently, increased sampling will correct for long-branch problems caused by widely divergent taxa (Donoghue et al., 1992; Olmstead et al., 1992; Chase et al., 1993; Qiu et al., 1993). However, in preliminary analyses of the ranunculids (including a number of Papaverales and the two genera comprising the Circaeasteraceae) based on *rbc*L data, the increased sampling does not move *Sinofranchetia* into the Lardizabalaceae (similar analyses of *atp*B data continue to recognize a monophyletic Lardizabalaceae). Work in progress will focus on the addition of the potential outgroup genus, *Sargentodoxa*, as well as increased sampling in the vicinity of *Dicentra* (i.e., Fumariaceae, Hypecoaceae, and other Papaverales).

For the three data sets, all comparative indicators of evolutionary rate, such as the number of variable sites, informative characters, and three-state characters, g_1 values, and indices measuring homoplasy, indicate that 18S nrDNA is the most conserved, *atp*B is intermediate, and *rbc*L is the least conserved of the three genes (Table 3). Sequence divergence values between matched pairs of taxa also suggest an intermediate rate of nucleotide substitutions for *atp*B (Table 4), and this is consistent with preliminary results for other ranunculid and (lower) hamamelid families that we have examined (Hoot & Crane, unpublished data). We conclude that while *atp*B, 18S nrDNA, and *rbc*L sequences are all useful for phylogenetic reconstruction at higher taxonomic levels, there are substantial differences in the degree of conservation of nucleotides among the three genes. In large-scale surveys of divergent taxa, a combination of several sequence data sets seems likely to provide the best possibility of resolving both proximal and distal branching patterns.

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A COMPARISON OF ANGIOSPERM PHYLOGENIES FROM NUCLEAR 18S rDNA AND *rbc*L SEQUENCES[1]

Daniel L. Nickrent[2] *and Douglas E. Soltis*[3]

Abstract

To investigate the phylogenetic utility of entire, nuclear-encoded small-subunit (18S) ribosomal DNA sequences, we compared the rate of evolution and phylogenetic resolution of entire 18S sequences with those for the chloroplast gene *rbc*L using a suite of 59 angiosperms and 3 gymnosperms (*Gnetum*, *Ephedra*, and *Zamia*) as outgroups. For *rbc*L, 482 (33.6%) of the 1431 base positions were phylogenetically informative, whereas for 18S rDNA 341 (18.4%) of the 1853 positions were informative. Pairwise comparisons within the angiosperms show that *rbc*L is generally about three times more variable than 18S rDNA. However, because the 18S region is approximately 400 base pairs longer than *rbc*L, the ratio of the number of phylogenetically informative sites per molecule is only about 1.4 times greater for *rbc*L compared to 18S rDNA. Not only are sites more variable in *rbc*L than in 18S rDNA, but this variability is more evenly distributed over the length of *rbc*L. In contrast, 18S rDNA shows highly variable regions interspersed with regions of extreme conservation. Minimum-length Fitch trees were constructed for each matrix, and the results were compared to a tree derived from a previous global analysis of *rbc*L sequences based on 499 seed plants. Parsimony analyses showed that several clades are strongly supported by both data sets, such as Gnetales, monocots, paleoherbs, Santalales, and various clades within Rosidae s.l. and Asteridae s.l. Some clades (e.g., Santalales) have higher base substitution rates for 18S rDNA, permitting the assessment of inter- and intrafamilial relationships. This comparative study indicates that 18S rDNA sequences contain sufficient information to conduct phylogenetic studies at higher taxonomic levels (family and above) within angiosperms. rDNA sequences are best applied to such deep divergences, but the amount of variation differs significantly among taxonomic groups.

The major morphologically based angiosperm classifications proposed during the past 15 years show marked similarities, yet also differ in fundamental ways. Recently, systematists have explored the utility of both DNA and RNA sequencing to resolve higher-level relationships within the angiosperms, as well as seed plants in general (e.g., Hamby & Zimmer, 1992; Chase et al., 1993). The largest molecular phylogenetic study conducted to date (Chase et al., 1993) employed sequence data for the chloroplast gene *rbc*L and was based on sequences for 499 species of angiosperms and other seed plants. The gene *rbc*L is typically 1428 base pairs in length, and the advantages of using this gene in phylogenetic reconstruction have been thoroughly reviewed (e.g., Ritland & Clegg, 1987; Palmer et al., 1988; Chase et al., 1993). These advantages include easy amplification via the polymerase chain reaction, essentially no insertion-deletion events, appropriate length and base substitution rate for inferring phylogeny at higher levels, and the availability of a set of sequencing primers (provided free of charge by G. Zurawski). Although some variation in the rate of *rbc*L sequence evolution occurs from lineage to lineage (Bousquet et al., 1992; Gaut et al., 1992), unequal rates of evolution do not appear to be sufficient to obscure major phylogenetic relationships (Chase et al., 1993). Because of these numerous advantages, *rbc*L sequences now exist for over 1500 taxa (M. Chase, pers. comm.), making *rbc*L the most frequently sequenced protein-coding gene. During the past several years, the phylogenetic analysis of *rbc*L sequences has provided unprecedented insights into higher-level relationships in angiosperms and gymnosperms (e.g., Chase et al., 1993; Conti et al., 1993; Duvall et al., 1993; Michaels et al., 1993; Morgan & Soltis, 1993; Qiu et al., 1993).

Because most evolutionary studies are devoid of positive controls to prove or disprove particular events, the strongest support that can be obtained in phylogenetic reconstruction is congruence re-

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sulting from analysis of multiple independent data sets (Miyamoto & Cracraft, 1991). Hypotheses of relationships are either strongly or weakly supported based upon statistical tests involving the data themselves (e.g., consistency index, bootstrap values, decay values). The more numerous and more varied the data sets that corroborate a given relationship, the greater the support for that relationship. Although *rbc*L and, more recently, other chloroplast genes such as *mat*K (see Johnson & Soltis, 1995, this issue) and *ndh*F (see Olmstead & Reeves, 1995, this issue) have been shown to have great utility in phylogeny estimation, many workers have emphasized the need for comparison of chloroplast DNA-based phylogenetic trees with those from other sources, especially those based on sequences from nuclear-encoded genes (e.g., Palmer, 1985; Rieseberg & Soltis, 1991; Doyle, 1992; Friedlander et al., 1992; Chase et al., 1993). At lower taxonomic levels (genus and below) comparative sequencing of the nuclear internal transcribed spacer (ITS) region has shown tremendous potential for inferring phylogenies (see Baldwin et al., 1995, this issue) and has stimulated the comparison of phylogenies based on chloroplast and nuclear DNA. At higher taxonomic levels, the phylogenetic trees presented for angiosperms based on *rbc*L sequences (Chase et al., 1993) have similarly stimulated interest in conducting a comparable phylogenetic analysis based on nuclear gene sequences. In this paper we explore the utility of entire nuclear 18S rDNA sequences for inferring phylogeny at higher levels within the angiosperms.

In plants, ribosomes exist in the chloroplasts, mitochondria, and cytoplasm and are composed of a small and a large subunit, each of which contains rRNA and associated proteins. Although sedimentation coefficients vary slightly, plant nuclear small-subunit rRNA will be referred to here as the 18S rRNA. The 18S, 5.8S, and 26S nuclear rRNA genes occur as a unit (cistron) separated by spacer regions. These cistrons are repeated hundreds to thousands of times in tandem arrays within the genome (Appels & Honeycutt, 1986). Ribosomal RNA cistrons are usually located in the nucleolar organizing region of the nucleus and may reside on several different chromosomes in plants (Thompson & Flavell, 1988). Sequence similarity between the individual cistrons within a single organism is extremely high, possibly due to unequal crossing over during meiosis, gene conversion, slippage, transposition, and RNA-mediated changes (Arnheim et al., 1980; Dover, 1982; Arnheim, 1983; Dover, 1987). The homogeneity of ribosomal RNA cistrons has been referred to as concerted evolution (Brown et al., 1972; Arnheim et al., 1980; Zimmer et al., 1980). Ribosomal loci represent an extreme type of concerted evolution (with essentially complete homogenization), making them advantageous for reconstruction of deep phylogenetic events (Sanderson & Doyle, 1993). Recent summaries of ribosomal RNA structure, function, gene organization, and evolution have been presented (Jorgansen & Cluster, 1988; Hillis & Dixon, 1991; Hamby & Zimmer, 1992).

Numerous low-molecular-weight (5S and 5.8S) rRNA sequences now exist (see compilation by Specht et al., 1991), and attempts have been made to use these sequences in addressing the origin and evolution of green plants (Hori et al., 1985; Hori & Osawa, 1987). However, because these molecules are less than 200 bp in length, they provide a very limited number of phylogenetically informative sites; hence, large numbers of equally parsimonious solutions often result when conducting studies using many taxa (Bremer et al., 1987). Specifically addressing 5S rRNA sequences, Mishler et al. (1988) summarized concerns for the use of rRNA sequences for phylogenetic reconstruction that apply to the 18S and 26S as well, such as cosubstitution in stem regions of helices, transition/transversion bias, alignment problems, different evolutionary rates, and homoplasy.

Both large- and small-subunit ribosomal RNA sequences have been used to examine the very deepest branches among organisms, such as the domains Eukarya, Bacteria, and Archae (Wolters & Erdmann, 1986; Olsen, 1987; Woese, 1987). Ribosomal RNA sequence data have also been used to elucidate phylogenetic relationships in animals (e.g., Sogin et al., 1986; Field et al., 1988; Wada & Satoh, 1994), protozoa (Schlegel et al., 1991), algae (Bhattacharya & Druehl, 1988; Buchheim et al., 1990; Huss & Sogin, 1990; Kantz et al., 1990; Hendriks et al., 1991; Chapman & Buchheim, 1991), and fungi (Förster et al., 1990; Swann & Taylor, 1993). Prior to 1990, most rRNA sequences were being determined from cloned material or by using Sanger dideoxynucleotide reactions and reverse transcriptase with rRNA templates (Lane et al., 1985). During the past several years, most workers have moved to direct sequencing of ribosomal DNA (rDNA) amplified via the polymerase chain reaction (PCR; Mullis & Faloona, 1987). The major reasons for the shift to DNA sequencing are: (1) rRNA is labile to RNases, making it methodologically difficult to extract, purify, and store; (2) rRNA secondary structure causes polymerase stalling, visualized as "hard stops" on sequencing gels, resulting in ambiguous sequence;

(3) when RNA is extracted from a tissue sample only RNA genes can be sequenced, whereas, theoretically, any gene (nuclear, plastid, mitochondrial) is available from total genomic DNA extracts; (4) DNA is easier to extract and is more stable than RNA; and (5) with DNA, both strands are available for sequencing, allowing more complete coverage of the molecule (as well as an opportunity to double-check each base position) by using primers designed for both the coding and noncoding strands.

The first 18S ribosomal RNA sequences of angiosperms were of rice (Takaiwa et al., 1984), maize (Messing et al., 1984), and soybean (Eckenrode et al., 1985). Later, the complete 18S rDNA sequence of the cycad *Zamia pumila* was published; with the above three higher plants and several outgroups, a phylogenetic tree was produced (Nairn & Ferl, 1988). Seven additional (partial) 18S rRNA sequences of members of the Poaceae were later determined, and a phylogenetic analysis of this family was conducted (Hamby & Zimmer, 1988).

Phylogenetic relationships in the parasitic plant order Santalales were examined by Nickrent & Franchina (1990). This study was the first since the work by Nairn & Ferl (1988) to use essentially complete 18S rRNA sequences in a phylogenetic analysis. Sequences from 13 angiosperm species representing 10 families were analyzed, and one most parsimonious tree was obtained that supported the monophyly of the order Santalales, confirmed the basal position of Olacaceae within the order, and showed Viscaceae to be derived from Santalaceae. This study indicated that sufficient information exists in complete 18S rRNA sequences to allow phylogenetic comparisons to be made at the family level and above.

Despite the phylogenetic promise of these initial analyses, relatively few 18S rRNA sequences were determined in the years that followed, perhaps due in part to the tremendous interest in *rbc*L sequencing for inferring phylogeny at this same level. As a result, the phylogenetic potential of 18S sequence data remained unexplored. A few entire sequences were published, including *Alnus glutinosa* (Savard & Lalonde, 1991), *Arabidopsis thalliana* (Unfried et al., 1989), *Lycopersicon esculentum* (Kiss et al., 1989), and *Sinapis alba* (Rathgeber & Capesius, 1990). A sequence for *Fragaria* × *ananassa* (Simovic et al., 1992) exists, but contains a large number of base changes atypical of other Rosaceae (and was therefore not included in the present study). Several studies, however, explored the phylogenetic potential of partial 18S sequences. For example, Troitsky et al. (1991) used five different rRNA molecules (including nuclear 18S rRNA) to examine the early evolution of seed plants. For 18S rRNA, 21 sequences representing 256 bp (from position 499 to 755 on *Glycine*) were used. Six dicots and eight monocots were included, as were *Ephedra* and *Gnetum*, two cycads, two gymnosperms (*Podocarpus* and *Taxus*), and *Lycopodium*. Two conclusions of this study were that the divergence of angiosperms from gymnosperms occurred before the early Carboniferous, i.e., at least 360 million years before present, and that the Gnetopsida are not monophyletic. Given the small number of base pairs used and that no statistical support for the clades was provided, these results must be viewed with caution. More recently, Chaw et al. (1993) used 18S rDNA sequence data to demonstrate support for the placement of *Taxus* in Coniferales; however, only four sequences (for *Taxus*, *Pinus*, *Podocarpus*, and *Ginkgo*) and an outgroup (*Zamia*) were used in the analysis.

By far the largest analyses of 18S sequences have been undertaken by Zimmer and her collaborators, who conducted phylogenetic studies using direct sequencing of rRNA from approximately 60 vascular plant species (Zimmer et al., 1989; Hamby & Zimmer, 1992). Their efforts toward producing a molecular phylogeny of the angiosperms were based on the sequencing of a portion of the small- (18S) and large- (26S) subunit rRNA molecules. Hamby & Zimmer (1992) used a total of 1701 base positions per taxon (1097 base positions from the 18S region and 604 positions from the 26S region) in a phylogenetic analysis of seed plants that included 29 dicot and 17 monocot genera. Two shortest trees were found with a large number of equally parsimonious solutions one or several steps longer. The shortest trees had a number of features in accord with various existing classifications, such as the presence of a monophyletic Gnetales clade as sister to the angiosperms, and the basal position within the angiosperms of several Magnoliid groups, such as Nymphaeaceae and Piperaceae. Sampling within nonmagnoliid groups was sparse, however, which could explain the unusual relationships suggested among more derived angiosperms (e.g., the presence of a clade composed of *Ranunculus*, *Duchesnea*, *Spinacia*, and *Stellaria*). Because many of the interior and basal dicot nodes were poorly supported in the rRNA tree, systematists remained unsure of the utility of ribosomal RNA sequences for resolving questions of angiosperm phylogeny. More recently, relationships among the tribes of Onagraceae were examined by Bult & Zimmer (1993) using partial

sequences of 18S and 26S rRNA. Although relatively few phylogenetically informative sites were found, several relationships were in accord with those revealed by *rbc*L analysis (Conti et al., 1993).

Using the same primers as the Zimmer group, Martin & Dowd (1991) obtained partial 18S and 26S rRNA sequences for 12 angiosperm species from 7 families. Their purpose was to assess the relative merits of phylogeny estimation using ribosomal sequences with those derived from *rbc*L. The authors concluded "both phylogenetic trees gave good grouping within families but in neither case was there resolution of the branching order of major taxa. . . ." The authors further stated "that neither macromolecule alone was likely to yield a solution to the problem of angiosperm phylogeny and therefore that studies of both, at least, will be required." However, aside from two familial placeholders, only two species (maize and rice) were shared by the two data sets; furthermore, taxon density was clearly a limitation in making any statements regarding the branching order of major taxa. Additional studies using partial 18S rRNA sequences include analyses of six angiosperm families (Boulter & Gilroy, 1992), Papilionaceae (Martin & Dowd, 1993), and Byblidaeae–Roridulaceae (Conran & Dowd, 1993). The latter study examined the phylogenetic placement of two carnivorous genera, *Roridula* and *Byblis*, that have been variously classified using morphological and chemical characters. The analysis of partial 18S rRNA sequences from these genera and 26 other angiosperms supported the position of *Roridula* in the lower Rosidae and the placement of *Byblis* in the Asteridae, results in agreement with *rbc*L sequence analysis (Albert et al., 1992).

The major goal of this study was to explore in more detail the potential of entire 18S rDNA sequences for inferring phylogeny at higher levels in the angiosperms. We wanted to understand better the rate of evolution and distribution of base substitutions of 18S rDNA compared to the widely used chloroplast gene *rbc*L. To accomplish these objectives, comparable 18S and *rbc*L sequence data sets were constructed for a similar suite of 59 angiosperms and 3 gymnosperms. Minimum-length Fitch trees were constructed, and relationships as well as evolutionary rates were compared for the entire 18S gene and *rbc*L. These phylogenetic analyses were not meant to be exhaustive studies of angiosperm relationships; we recognize that taxonomic density is a limitation in this study. Rather, our goal was to assess the relative merits and attributes of each molecule through direct comparison. This study differs from previous comparative analyses of 18S and *rbc*L sequences (e.g., Martin & Dowd, 1991) in that essentially complete sequences of both genes were used, taxon sampling was more extensive, and, in the majority of cases, the same taxon was sequenced for both genes.

Materials and Methods

Taxon sampling and sequence acquisition

Given the large, taxonomically diverse array of *rbc*L sequences that is already available, taxon inclusion for this study was determined mainly by the availability of 18S rRNA or rDNA sequences. We therefore determined the 18S rDNA sequences of additional plant taxa to provide greater coverage of the major clades identified in the global *rbc*L analysis of Chase et al. (1993). The *rbc*L sequences were chosen to correspond at the specific or, secondarily, the generic level to an available sequence of 18S rRNA. Some *rbc*L sequences included in Chase et al. (1993), but not deposited in Genbank, were kindly provided by Mark Chase (*Brassica*, *Pachysandra*, *Pisum*, and *Impatiens*). An *rbc*L sequence of *Hydrocotyle* that was not included in the Chase et al. analysis was also kindly provided by G. Plunkett. The original *rbc*L sequence for *Zea* contained errors; hence the newly determined sequence (Gaut et al., 1992) was used herein. In addition to the three published *rbc*L sequences of Santalales (Morgan & Soltis, 1993), 12 other *rbc*L sequences were determined to increase sampling within this one order, thereby allowing phylogeny comparison of more closely related species using both molecules. A data set of 62 taxa was ultimately identified for which sequences of both molecules were available for use in this study (Tables 1 and 2). Of the 62 sequence pairs (18S rDNA and *rbc*L) used herein, 37 were from the same species; an additional 15 sequences were from different species within the same genus. Different genera were used for ten families to allow a broader sampling within the angiosperms (*rbc*L/18S rDNA): *Betula*/*Alnus*, *Pachysandra*/*Buxus*, *Convolvulus*/*Cuscuta*, *Polemonium*/*Gilia*, *Pisum*/*Glycine*, *Lambertia*/*Knightia*, *Reinwardtia*/*Linum*, *Byrsonima*/*Malpighia*, *Pyrola*/*Monotropa*, and *Mahonia*/*Podophyllum*. Although members of three of the above generic pairs are classified in separate families (i.e., Cuscutaceae/Convolvulaceae, Monotropaceae/Pyrolaceae, Podophyllaceae/Berberidaceae), they were deemed to be related closely enough (based upon traditional classifications) to be used as placeholders. Following Cronquist (1981), 47 angiosperm families are represented in this study.

Seven of the 62 18S rDNA sequences used in

TABLE 1. Voucher information for 18S rDNA sequences.

Species*	Family	Voucher	Reference† (source)	Sequence	GenBank number
Acorus calamus L.	Araceae	Nickrent 2942, SIU	†(Nickrent)	6–1761	L24078
Akebia quinata (Houtt.) Decne.	Lardizabalaceae	Nickrent 2945, SIU	†(Nickrent)	66–1705	L31795
Alnus glutinosa (L.) Gaertn.*	Betulaceae	unknown	Savard & Lalonde, 1991	79–1782	X54984
Antidaphne viscoidea (Griseb.) Kuijt	Eremolepidaceae	Nickrent 2730, SIU	†(Nickrent)	6–1784	L24080
Arceuthobium verticilliflorum Englem.	Viscaceae	Nickrent 2065, SIU	Nickrent & Starr, 1994	10–1808	L24042
Aristolochia tomentosa Sims.	Aristolochiaceae	Nickrent 2922, SIU	†(Nickrent)	5–1765	L24083
Asarum canadense L.	Aristolochiaceae	Nickrent 2888, SIU	Nickrent & Starr, 1994	6–1761	L24043
Brassica hirta Moench [= *Sinapis alba* L.]	Brassicaceae	unknown	Rathgeber & Capesius, 1990	1–1804	X17062
Buxus sempervirens L.*	Buxaceae	Nickrent 2890, SIU	Nickrent & Franchina, 1990	2–1747	X16599
Chrysosplenium iowense Rydb.	Saxifragaceae	Wendel *s.n.*, ISC	†(Soltis)	23–1778	L28136
Cornus florida L.	Cornaceae	Nickrent 2897, SIU	Nickrent & Franchina, 1990	3–1757	X17370
Cuscuta gronovii Willd.*	Cuscutaceae	Nickrent 3015, SIU	†(Nickrent)	6–1764	L24747
Dendrophthora clavata (Benth.) Urban	Viscaceae	Nickrent 2182, SIU	†(Nickrent)	5–1765	L24086
Drimys winteri Forster & Forster	Winteraceae	Nickrent 2951, SIU	†(Nickrent)	9–1763	L24089
Ephedra antisyphilitica BErl. ex C. A. May	Ephedraceae	Nickrent 2995, SIU	†(Nickrent)	11–1767	L24091
Eubrachion ambiguum (Hooker & Arnott) Engl.	Eremolepidaceae	Nickrent 2699, SIU	†(Nickrent)	4–1759	L24141
Euonymus alatus (Thunb.) Siebold	Celastraceae	Nickrent 2894, SIU	Nickrent & Franchina, 1990	3–1757	X16600
Francoa sonchifolia Cav.	Saxifragaceae	Soltis & Soltis 2479, WS	†(Soltis)	23–1764	L28137
Gaiadendron punctatum (R. & P.) G. Don	Loranthaceae	Nickrent 2729, SIU	†(Nickrent)	13–1761	L24143
Gilia capitata Sims.*	Polemoniaceae	Johnson 9215, WS	†(Soltis)	24–1770	L28138
Ginalloa arnottiana Korth.	Viscaceae	Beaman 9074, K	†(Nickrent)	7–1766	L24144
Glycine max (L.) Merrill*	Fabaceae	unknown	Eckenrode et al., 1985	1–1807	X02623
Gnetum leyboldii Tul.	Gnetaceae	Nickrent 2946, SIU	Nickrent & Starr, 1994	10–1812	L24045
Gossypium hirsutum L.	Malvaceae	Nickrent 2904, SIU	†(Nickrent)	13–1757	L24145
Hedera helix L.	Araliaceae	Nickrent 2887, SIU	Nickrent & Franchina, 1990	4–1759	X16604
Heuchera micrantha Dougl. ex Lindl.	Saxifragaceae	Soltis & Soltis 1949, WS	†(Soltis)	21–1778	X28139
Houttuynia cordata Thunb.	Saururaceae	Nickrent 2940, SIU	†(Nickrent)	6–1764	L24147
Hydrangea macrophylla (Thunb.) Ser.	Hydrangeaceae	Morgan 2150, WS	†(Soltis)	24–1528	L28140
Hydrocotyle sibthorpioides Lam.	Apiaceae	Nickrent 2886, SIU	†(Nickrent)	3–1735	X16605
Impatiens pallida Nutt.	Balsaminaceae	Nickrent 2938, SIU	†(Nickrent)	9–1758	L24148
Knightia excelsa R. Br.*	Proteaceae	Soltis *s.n.*	†(Nickrent)	6–1761	L24155
Korthalsella lindsayi (D. Oliv.) Engl.	Viscaceae	Nickrent 2740, SIU	†(Nickrent)	6–1710	L24150

TABLE 1. Continued.

Species*	Family	Voucher	Reference† (source)	Sequence	GenBank number
Lepuropetalon spathulatum (Muhl.) Elliott	Saxifragaceae	Thomas *s.n.*, NLU	†(Soltis)	23–1764	L28141
Linum perenne L.*	Linaceae	Nickrent 2900, SIU	†(Nickrent)	11–1746	L24401
Lycopersicon esculentum Mill.	Solanaceae	unknown	Kiss et al., 1989	1–1800	X51576
Malpighia coccigera L.*	Malpighiaceae	Nickrent 2903, SIU	Nickrent & Starr, 1994	15–1760	L24046
Monotropa uniflora L.*	Monotropaceae	Nickrent 3018, SIU	†(Nickrent)	6–1764	L26062
Morus alba L.	Moraceae	Nickrent 2924, SIU	†(Nickrent)	6–1759	L24398
Misodendron brachystachyum D.C.	Misodendraceae	Nickrent 2829, SIU	†(Nickrent)	5–1761	L24397
Notothixos subaureus Oliver	Viscaceae	Nickrent 2790, SIU	†(Nickrent)	5–1717	L24402
Nymphaea tuberosa Paine	Nymphaeaceae	Nickrent 2906, SIU	†(Nickrent)	1–1759	L24404
Nyssa sylvatica Marsh	Nyssaceae	Nickrent 2905, SIU	Nickrent & Franchina, 1990	3–1751	X16603
Opilia amentacea Roxb.	Opiliaceae	Nickrent 2816, SIU	†(Nickrent)	4–1760	L24407
Oryza sativa L.	Poaceae	unknown	Takaiwa et al., 1984	1–1812	X00755
Osyris lanceolata Hochst. & Steud.	Santalaceae	Nickrent 2731, SIU	†(Nickrent)	5–1801	L24409
Paeonia lactiflora Pall.	Paeoniaceae	Nickrent 2988, SIU	†(Nickrent)	6–1763	L24410
Peperomia serpens (Swartz) Loud.	Piperaceae	Nickrent 2907, SIU	†(Nickrent)	32–1761	L24411
Phoradendron serotinum (Raf.) M. C. Johnst.	Viscaceae	Nickrent 2077, SIU	Nickrent & Franchina, 1990	3–1769	X16607
Pittosporum japonicum Hort. ex C. Presl.	Pittosporaceae	Riesberg *s.n.*, RSA	†(Soltis)	39–1775	L28142
Podophyllum peltatum L.*	Berberidaceae s.l.	Nickrent 2891, SIU	†(Nickrent)	4–1766	L24413
Prunus persica (L.) Batsch.	Rosaceae	E. E. Dickson, Bailey Hort.	†(Soltis)	23–1778	L28749
Ranunculus sardous Crantz	Ranunculaceae	Nickrent 2932, SIU	†(Nickrent)	6–1761	L24092
Ribes aureum Pursh	Grossulariaceae	Soltis & Soltis 2220, WS	†(Soltis)	29–1770	L28143
Santalum album L.	Santalaceae	Nickrent 2734, SIU	†(Nickrent)	5–1764	L24416
Saruma henryi Oliver	Aristolochiaceae	Qiu 91018, NCU	†(Nickrent)	5–1765	L24417
Schoepfia schreberi Gemlin	Olacaceae	Nickrent 2599, SIU	†(Nickrent)	7–1758	L24418
Sparganium eurycarpum Engl.	Sparganiaceae	Nickrent 2943, SIU	†(Nickrent)	6–1749	L24419
Spinacia oleracea L.	Chenopodiaceae	Nickrent 2896, SIU	†(Nickrent)	8–1764	L24420
Tropaeolum majus L.	Tropaeolaceae	Chase 113, NCU	†(Soltis)	24–1776	L28750
Viscum album L.	Viscaceae	Nickrent 2253, SIU	†(Nickrent)	4–1762	L24426
Zea mays L.	Poaceae	unknown	Messing et al., 1984	1–1809	K02202
Zamia pumila L.	Zamiaceae	unknown	Nairn & Ferl, 1988	1–1813	M20017

† Sequence first reported here.
* Different genera used as placeholders for family for phylogenetic analyses.

TABLE 2. Voucher information for *rbc*L sequences.

Species	Family	Voucher	Reference (source)	Sequence	GenBank number
Acorus calamus L.	Araceae	French 232, CH	Duvall et al., 1993 (Duvall)	29–1270, 1319–1352	M91625
Akebia quinata (Houtt.) Decne.	Lardizabalaceae	Qiu 91020, NCU	Chase et al. 1993 (Qiu)	29–1428	L12627
Antidaphne viscoidea (Griseb.) Kuijt	Eremolepidaceae	Nickrent 2730, SIU	†(Nickrent)	4–1428	L26068
Arceuthobium verticilliflorum Engelm.	Viscaceae	Nickrent 2065, SIU	†(Nickrent)	3–1428	L26067
Aristolochia macrophylla Lam.	Aristolochiaceae	Qiu 91019, NCU	Qiu et al., 1993	29–1428	L12630
Asarum canadense L.	Aristolochiaceae	none	Chase et al., 1993 (Olmstead)	27–1428	L14290
Betula nigra L.*	Betulaceae	Chase 255, NCU	Albert et al., 1992 (Qiu)	27-1428	L01889
Brassica campestris L.	Brassicaceae	none	Olmstead et al., 1992 (Nugent & Palmer)	1–1428	—
Byrsonima crassifolia (L.) Kunth.*	Malpighiaceae	Fairchild Trop. G. 81680, MICH	Albert et al., 1992 (Chase)	29–1428	L01892
Chrysosplenium iowense Rydb.	Saxifragaceae	Wendel *s.n.*, ISC	†(Soltis)	16–1428	—
Convolvulus tricolor L.*	Convolvulaceae	none	Olmstead et al., 1992	103–1428	L11683
Cornus florida L.	Cornaceae	Xiang 250, WS	Xiang et al., 1993	27–1428	L11215
Dendrophthora clavata (Benth.) Urban	Viscaceae	Nickrent 2182, SIU	†(Nickrent)	3–1428	L26069
Drimys winteri Forster & Forster	Winteraceae	Qiu 90016, NCU	Albert et al., 1992 (Qiu)	29–1428	L01905
Ephedra tweediana C. A. Mey.	Ephedraceae	none	Chase et al., 1993 (Chase)	30–1428	L12677
Eubrachion ambiguum (Hooker & Arnott) Engl.	Eremolepidaceae	Nickrent 2699, SIU	†(Nickrent)	12–1428	L26071
Euonymus alatus (Thunb.) Siebold	Celastraceae	Chase 137, NCU	Chase et al,. 1993 (Chase)	27–1428	L13184
Francoa sonchifolia Cav.	Saxifragaceae	Soltis & Soltis 2479, WS	Soltis et al., 1990	31–1407	L11184
Gaiadendron punctatum (R. & P.) G. Don	Loranthaceae	Nickrent 2729, SIU	†(Nickrent)	4–1373	L26072
Ginalloa arnottiana Korth	Viscaceae	Nickrent 2982, SIU	†(Nickrent)	6–1428	L26070
Gnetum gnemon L.	Gnetaceae	Chase 208, NCU	Chase et al., 1993 (Chase)	26–1351	L12680
Gossypium robinsonii F. Muell.	Malvaceae	Wendel *s.n.*, ISC	Chase et al., 1993 (Chase)	27–1428	L13186
Hedera helix L.	Araliaceae	Jansen *s.n.*, MICH	Olmstead et al., 1993	27–1428	L01924
Heuchera micrantha Dougl. ex Lindl.	Saxifragaceae	Soltis & Soltis 1949, WS	Soltis et al., 1990	31–1407	L01925
Houttuynia cordata Thunb.	Saururaceae	Les *s.n.*, CONN	Chase et al., 1993 (Les)	29–1183	L08762
Hydrangea macrophylla (Thunb.) Ser.	Hydrangeaceae	Morgan 2150, WS	Morgan & Soltis, 1993	31–1407	L11187
Hydrocotyle sp.	Apiaceae	unknown	Chase (pers. comm.)	16–1407	—
Impatiens capensis Meerb.	Balsaminaceae	Chase 114, NCU	Chase et al., 1993 (Chase)	29–1428	—
Korthalsella lindsayi (D. Oliv.) Engl.	Viscaceae	Nickrent 2740, SIU	†(Nickrent)	13–1428	L26073
Lambertia inermis R. Br.*	Proteaceae	Nat. Trop. BG, Hawaii	Morgan & Soltis, 1993	31–1407	L11190
Lepuropetalon spathulatum (Muhl.) Elliott	Saxifragaceae	Thomas *s.n.*, NLU	Morgan & Soltis, 1993	31–1407	L11192
Lycopersicon esculentum Mill.	Solanaceae	none	Olmstead et al., 1993	1–1428	L14403

TABLE 2. Continued.

Species	Family	Voucher	Reference (source)	Sequence	GenBank number
Mahonia bealei (Fortune) Carr.*	Berberidaceae	Qiu 74, NCU	Qiu et al., 1993	29–1428	L12657
Morus alba L.	Moraceae	none	Albert et al., 1992 (Golenberg)	1–1407	L01933
Misodendron brachystachyum D.C.	Misodendraceae	Nickrent 2829, SIU	†(Nickrent)	5–1428	L26074
Notothixos subaureus Oliver	Viscaceae	Nickrent 2790, SIU	†(Nickrent)	15–1428	L26075
Nymphaea odorata Aiton	Nymphaeaceae	Les *s.n.*, CONN	Les et al., 1991	29–1183§	M77034
Nyssa ogeche Marsh	Nyssaceae	U.S. Natl. Arb. *s.n.*	Xiang et al., 1993	31–1407	L11228
Opilia amentacea Roxb.	Opiliaceae	Nickrent 2816, SIU	†(Nickrent)	3–1428	L26076
Oryza sativa L.	Poaceae	unknown	Nishizawa & Hirai, 1987	1–1428	D00207
Osyris lanceolata Hochst. & Steud.	Santalaceae	Nickrent 2731, SIU	Morgan & Soltis, 1993	31–1407	L11196
Pachysandra procumbens Michx.*	Buxaceae	Chase 207, NCU	Chase et al., 1993 (Chase)	58–1360	—
Paeonia tenuifolia L.	Paeoniaceae	Kron 2115, NCU	Chase et al., 1993 (Kron)	29–1428	L13687
Peperomia sp.	Piperaceae	Qiu 91047, NCU	Qiu et al., 1993	27–1428	L12661
Phoradendron serotinum (Raf.) M. C. Johnst.	Viscaceae	Nickrent 2077, ILL	Morgan & Soltis, 1993	31–1407	L11199
Pisum sativum L.*	Fabaceae	unknown	Zurawski et al., 1986	1–1428	—
Pittosporum japonicum Hort. ex C. Presl.	Pittosporaceae	Rieseberg *s.n.*, RSA	Morgan & Soltis, 1993	31–1407	L11202
Polemonium reptans L.*	Polemoniaceae	none	Olmstead et al., 1992	103–1428	L11687
Prunus virginiana L.	Rosaceae	Soltis & Soltis 2475, WS	Albert et al., 1992 (Morgan & Soltis)	31–1407	L01947
Pyrola rotundifolia L.*	Pyrolaceae	Kron 1906, NCU	Kron & Chase, 1993	29–1428	L12622
Ranunculus trichophyllus Chaix	Ranunculaceae	Les *s.n.*, CONN	Chase et al., 1993 (Les)	29–1183	L08766
Reinwardtia indica Dumort.*	Linaceae	Chase 230, NCU	Chase et al., 1993 (Chase)	27–1366	L13188
Ribes aureum Pursh	Grossulariaceae	Soltis & Soltis 2220, WS	Morgan & Soltis, 1993	31–1407	L11204
Santalum album L.	Santalaceae	Nickrent 2734, SIU	†(Nickrent)	7–1428	L26077
Saruma henryi Oliver	Aristolochiaceae	Qiu 91018, NCU	Qiu et al., 1993	29–1428	L12664
Schoepfia schreberi Gmelin	Olacaceae	Nickrent 2599, SIU	Morgan & Soltis, 1993	30–1428	L11205
Sparganium americanum Engl.	Sparganiaceae	Chase 257, NCU	Duvall et al., 1993 (Duvall)	20–1351	M91633
Spinacia oleracea L.	Chenopodiaceae	unknown	Zurawski et al., 1981	1–1428	J01443
Tropaeolum majus L.	Tropaeolaceae	Chase 113, NCU	Price & Palmer 1993	27–1428	L14706
Viscum album L.	Viscaceae	Nickrent 2253, SIU	†(Nickrent)	13–1428	L26078
Zea mays L.	Poaceae	unknown	Gaut et al., 1992	1–1428	Z11973
Zamia inermis Vovides, Reese & Vásquez-Torres	Zamiaceae	Schutzman *s.n.*, FLAS	Chase et al., 1993 (Hills & Chase)	31–1428	L12683

† Sequence first reported here.
* Different genera used as placeholders for family for phylogenetic analyses.
§ The remaining sequence (1184–1428) was not available through Genbank, but was determined by Qiu (Chase et al., 1993) and sent by Mark Chase.

this study were previously published by workers other than the authors (Table 1). With the exception of six sequences that were obtained via direct sequencing of rRNA using reverse transcriptase (Nickrent & Franchina, 1990), all of the remaining rDNA sequences were derived from PCR products.

AMPLIFICATION AND SEQUENCING

The genomic DNAs used for amplification and sequencing of 18S rDNA and *rbc*L were extracted using a modification of the hot CTAB method (Doyle & Doyle, 1987; Nickrent, 1994). Plant samples were derived from either fresh, silica gel-dried, or herbarium material. The PCR protocols employed, as well as the oligonucleotide primers used for the amplification and subsequent sequencing of rDNA, are provided in Nickrent (1994) and Nickrent & Starr (1994). The general PCR strategy and amplification primers used for *rbc*L are provided in Morgan & Soltis (1993). For both *rbc*L and 18S rDNA, the first author employed direct sequencing of double-stranded PCR products. These products were prepared for sequencing by gel purification whereby the PCR bands are bound to DEAE membranes, eluted, and precipitated in ethanol. DNA so prepared is denatured at 100°C and snap-cooled for primer annealing. In contrast, the second author used each of the two PCR primers individually to generate single-stranded DNA from the double-stranded PCR products. Single-stranded 18S and *rbc*L DNAs were then purified by precipitation with 20% PEG/2.5 M NaCl, as described by Morgan & Soltis (1993). In all instances, the chain-termination method of sequencing was employed (Sanger et al., 1977) using [^{35}S] dATP and the Sequenase® version 2.0 kit. DNA fragments were separated in 6% acrylamide gels; gels were subsequently fixed and used to expose film using standard techniques. Compressions and other structure-related artifacts were resolved either through the use of alternative nucleotides (deaza-dGTP, dITP) or by sequencing the same region on the complementary strand.

SEQUENCE FEATURES AND MULTIPLE ALIGNMENTS

All alignments were initially conducted on a SUN Spark Station running the Genetic Data Environment (GDE, version 2.2; Smith, 1992). These alignments were downloaded to a Macintosh computer and directly imported into MacClade (version 3.01; Maddison & Maddison, 1992). For each molecule, the chart features of MacClade were used to examine patterns of variability and conservation, transition/transversion bias, and (for *rbc*L) the number of changes per codon position. This program was also used to determine the number of phylogenetically informative sites for each alignment. MacClade files were saved in Nexus format and then imported into PAUP (version 3.1; Swofford, 1993) for parsimony analyses. Files containing the complete alignments of both molecules are available from both authors by sending a formatted 3.5-inch diskette.

The alignment of *rbc*L sequences is straightforward and can be accomplished easily by sight because very few length mutations exist. In contrast, rDNA sequence alignment was performed as an iterative process that simultaneously dealt with phylogenetic relationships, compensatory mutations, and higher-order structure. The higher-order rRNA structure of *Glycine max* (Fig. 1), like the recently proposed structure for *Rafflesia keithii* (Nickrent & Starr, 1994), is similar to the one given for maize by Gutell et al. (1985) but includes the structural changes proposed for yeast (Gutell, 1993) and eukaryotes (Neefs et al., 1993). This structure differs somewhat from that proposed by Senecoff & Meagher (1992), which was based largely on a mammalian model. The soybean structure given here was used as a reference for examining structural variation in the other plant species examined and as a guide during the alignment process.

Until recently, the secondary structure of rRNA made direct sequencing of this molecule very difficult and aroused some criticism over the utility of rRNA for phylogenetic reconstruction in plants. With the advent of PCR, the amplification and sequencing of rDNA is no more difficult than for other genes (Nickrent & Starr, 1994). Furthermore, secondary structure provides much-needed corroboratory information regarding base pairing and compensatory base changes that is essential in producing alignments that reflect proper base homology.

The 18S rDNA sequences obtained were approximately 1770 base pairs in length. Published

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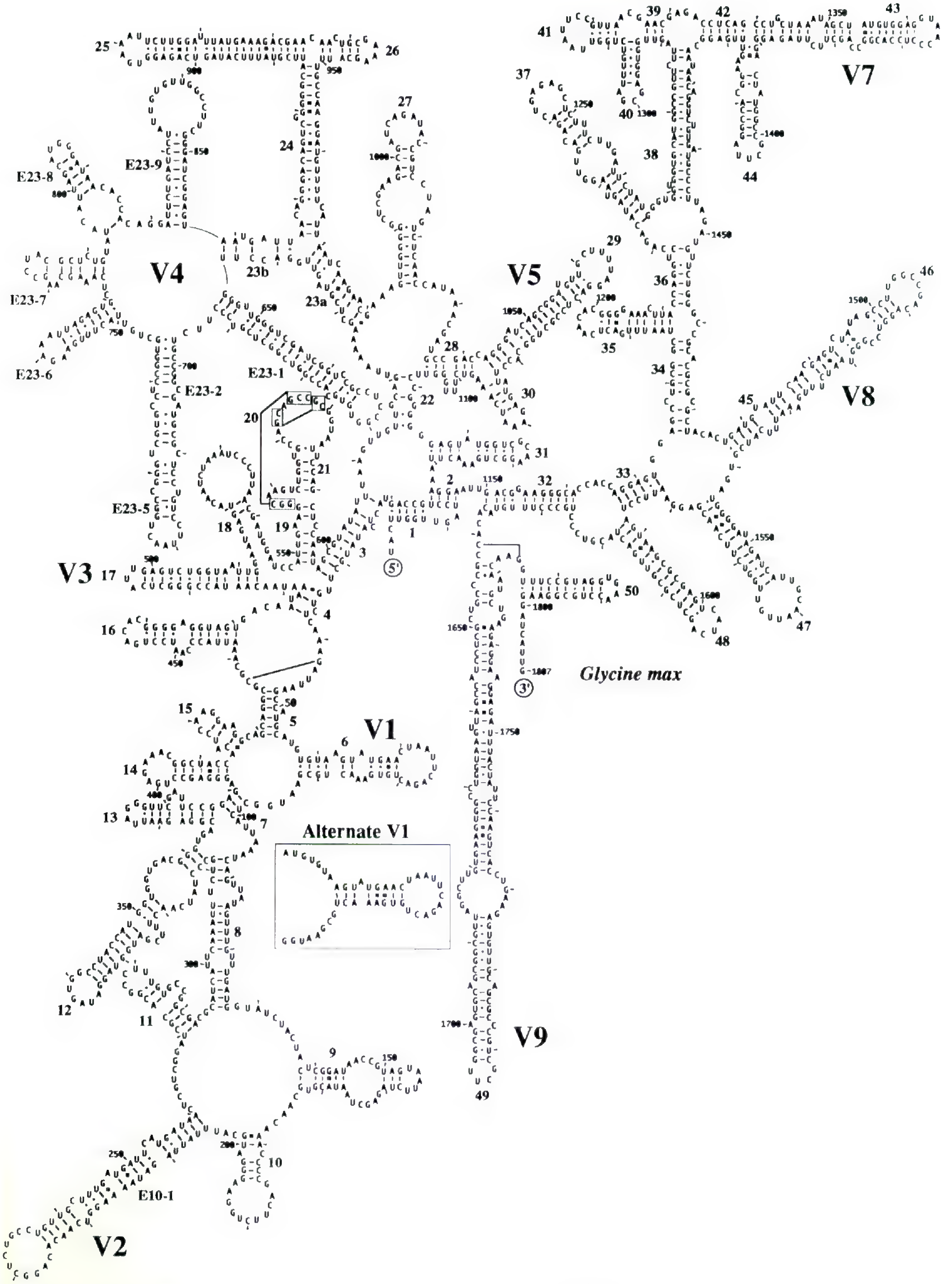
FIGURE 1. Proposed secondary structural model for the small-subunit (18S) ribosomal RNA of *Glycine max*. The primary sequence of soybean was determined by Eckenrode et al. (1985). This structural model follows the general models proposed by Gutell et al. (1985) for *Zea*, Gutell (1993) for *Saccharomyces*, and Neefs et al. (1993) for



eukaryotes in general. Helix numbering corresponds to Neefs et al. (1993). The structure for helix 6 (V1 region) follows Gutell (1993), with the alternative interpretation according to Neefs et al. (1993). The structure for the V4 region follows Nickrent & Sargent (1991). For an alternative model of the V4 involving a pseudoknot between helices E23-8 and E23-9, see Neefs et al. (1993). The V6 region is absent in eukaryotes. Tertiary interactions are indicated by thick lines.

complete 18S rDNA sequences of higher plants vary in length from 1800 to 1813 base pairs (mean of 1807 base pairs); thus, in this study, 97.9% of the total length of the molecule was obtained for most taxa. Owing to alignment spacers ("-"), the total length of the matrix was 1853. Insertion/deletion events (indels) were treated as missing data. Certain regions of 18S rDNA are variable in primary sequence and length, such as the termini of helices E10-1, E23-1, and 49 (Fig. 1). These regions confound unambiguous alignment; hence positions 227–239 and 676–685 on the alignment (equivalent to sites 224–232 and 664–673 on the *Glycine* molecule) were eliminated from analysis as suggested by Swofford & Olsen (1990). Those base pairs corresponding to the 25e forward 18S rDNA PCR primer (positions 1–20) were removed from the analysis. Similarly, those base pairs corresponding to the 1769 reverse PCR primer (sites 1810–1853 on the alignment, 1764–1807 on *Glycine*) were eliminated. With the exception of the excluded base pairs, alignment of the 18S rDNA sequences was straightforward because most length mutations involve single base insertions or deletions.

The total length of the *rbc*L data matrix was 1431. However, the first 30 base pairs were not used, because, after amplification, this portion of the gene is identical to the Z1 forward amplification primer. Sequences of *Nymphaea*, *Houttuynia*, and *Ranunculus* were incomplete (see Table 2); "?" was used to indicate missing sites.

PHYLOGENETIC ANALYSES

Minimum-length Fitch parsimony trees were constructed using PAUP version 3.1.1 (Swofford, 1993) with MULPARS and TBR branch swapping. Given the number of taxa (62), only heuristic search strategies could be employed. Both data sets were analyzed giving all changes equal weight. Trials using the character-state transformation weighting model of Albert et al. (1993) for the *rbc*L matrix and a transformation matrix encompassing a 10:1 bias favoring transitions over transversions for the 18S rDNA data gave similar results as trials with equal weighting. To determine whether multiple, equally parsimonious "islands" of most parsimonious trees exist (Maddison, 1991), 100 replicate searches with random taxon addition were conducted. To obtain estimates of reliability for monophyletic groups, bootstrap (Felsenstein, 1985) analyses (100 replicates) were conducted. For both data sets, the bootstrap analysis was performed using simple taxon addition, TBR branch swapping, ACCTRAN character-state optimization, and unweighted characters.

The phylogenetic trees derived from the global *rbc*L analyses of Chase et al. (1993) were used to construct a "reference tree" for the subset of taxa used in this study (Fig. 2). This reference tree was also constructed to assess the effect of taxon sampling and density on the stability and composition of various clades as determined by the present analyses. The Search II strategy employed by Chase et al. (1993) resulted in 3900 shortest trees, one of which was chosen at random and depicted in their figure 2B. Search II was preferred by the authors (over their Search I) because it included a greater diversity of taxa, was able to save more trees of shortest length, and did not use relative weighting of character-state transformations. In figure 2B of Chase et al. (1993), the angiosperms were divided into 19 major groups, the composition of which varied from single genera to groups of many families. Owing to lack of an 18S rDNA sequence, the reference tree constructed here does not include four of the major *rbc*L clades: *Ceratophyllum*, *Gunnera*, Laurales, and Asterid V. The Asterid V clade was not in the Search II topology of Chase et al. (1993), but Asterid V included Santalales in the tree resulting from Search I. It is well-known that sampling affects tree topologies (Felsenstein, 1985); the reference tree of Figure

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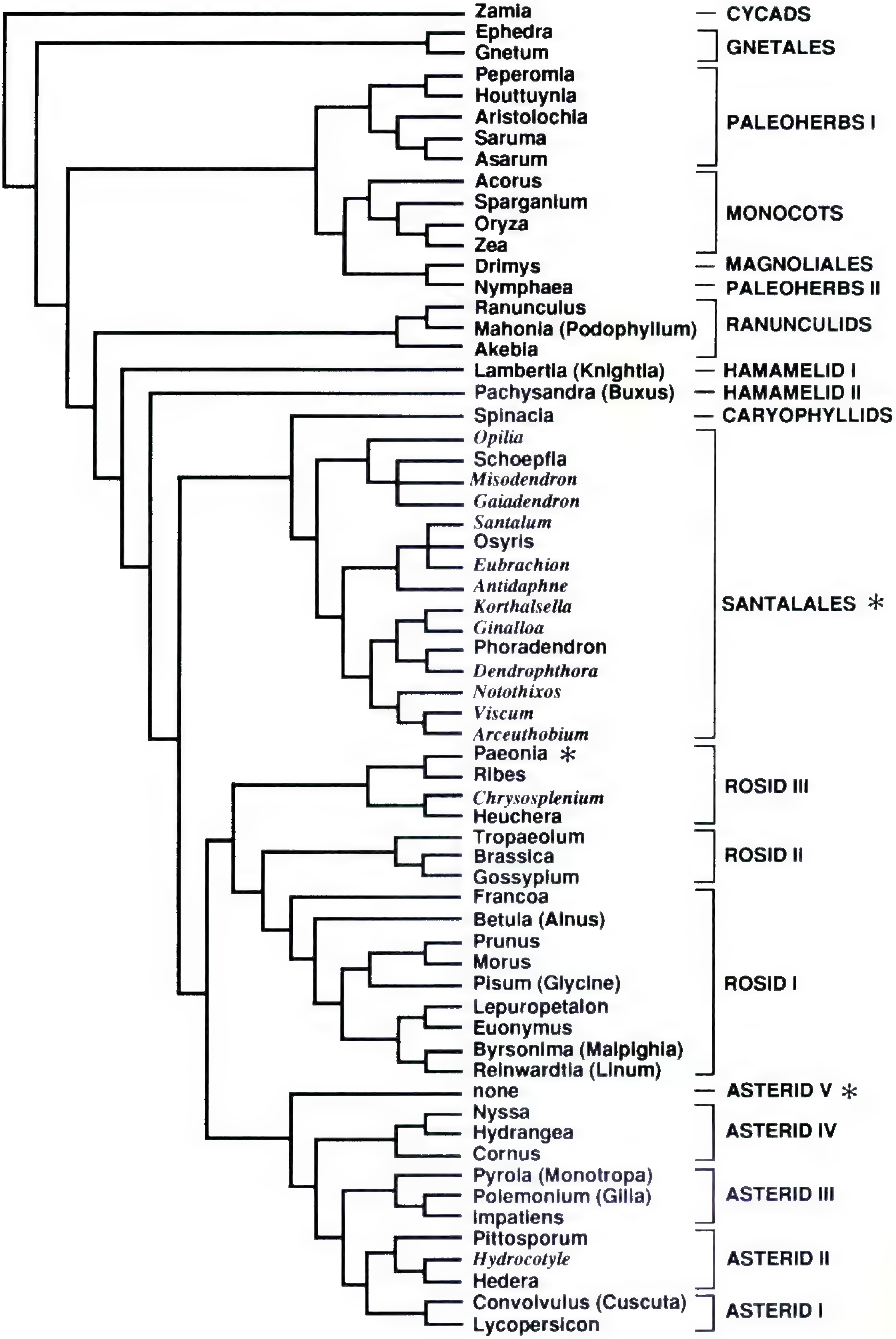
FIGURE 2. The "reference tree" constructed from the topology found in tree 2B in the global *rbc*L analysis that included 499 taxa (Chase et al., 1993). This tree represents a null hypothesis that assumes *rbc*L is insensitive to taxon sampling, i.e., all topologies using fewer taxa are fully concordant with the global topology. Instances where two generic names are given represent cases where different generic representatives of a family were used (*rbc*L taxon first followed by 18S rDNA taxon in parentheses). Those taxa in italics were not included in the study by Chase et al. (1993); their placement on the tree is derived from the analysis reported here (e.g., Santalales) or based upon traditional familial classifications (e.g., *Hydrocotyle*, Apiaceae). The names of the major angiosperm clades (right side) correspond to Chase et al. (1993). Taxa marked with an asterisk (*, Santalales and *Paeonia*) were located on the Asterid V clade in Search I of Chase et al. (1993).


Zamia
Ephedra
Gnetum
Peperomia
Houttuynia
Aristolochia
Saruma
Asarum
Acorus
Sparganium
Oryza
Zea
Drimys
Nymphaea
Ranunculus
Mahonia (Podophyllum)
Akebia
Lambertia (Knightia)
Pachysandra (Buxus)
Spinacia
Opilia
Schoepfia
Misodendron
Gaiadendron
Santalum
Osyris
Eubrachion
Antidaphne
Korthalsella
Ginalloa
Phoradendron
Dendrophthora
Notothixos
Viscum
Arceuthobium
Paeonia *
Ribes
Chrysosplenium
Heuchera
Tropaeolum
Brassica
Gossypium
Francoa
Betula (Alnus)
Prunus
Morus
Pisum (Glycine)
Lepuropetalon
Euonymus
Byrsonima (Malpighia)
Reinwardtia (Linum)
none
Nyssa
Hydrangea
Cornus
Pyrola (Monotropa)
Polemonium (Gilia)
Impatiens
Pittosporum
Hydrocotyle
Hedera
Convolvulus (Cuscuta)
Lycopersicon
CYCADS
GNETALES
PALEOHERBS I
MONOCOTS
MAGNOLIALES
PALEOHERBS II
RANUNCULIDS
HAMAMELID I
HAMAMELID II
CARYOPHYLLIDS
SANTALALES *
ROSID III
ROSID II
ROSID I
ASTERID V *
ASTERID IV
ASTERID III
ASTERID II
ASTERID I

2 should therefore be interpreted as a null hypothesis that assumes *rbc*L is insensitive to taxon inclusion and that the topology of a restricted analysis is congruent with that of a global analysis.

Results

General features of *rbc*L and 18S rDNA

The length of *rbc*L is highly conserved in higher plants with few insertion/deletion events reported (Chase et al., 1993). Positions 1426–1428 form the most common stop codon, although longer reading frames up to 1458 bp have been reported in Asteraceae (Kim et al., 1992). Among the taxa analyzed herein, a single insertion of three bases occurs in *Zea* beginning at position 1404, whereas all other full length *rbc*L sequences used herein are of length 1428. For *rbc*L, 482 (33.6%) of the 1431 base positions are potentially phylogenetically informative. The length of complete 18S rDNA also varies: 1800 bp (*Lycopersicon*), 1804 bp (*Brassica*), 1807 bp (*Glycine*), 1809 bp (*Zea*), 1812 bp (*Oryza*), and 1813 bp (*Zamia*). Of the 1853 positions for the 18S rDNA alignment, 341 (18.4%) are potentially phylogenetically informative.

When one compares sequences of two distantly related taxa, for example, *Zamia* and *Pisum* (Fabaceae) for *rbc*L and *Zamia* and *Glycine* (Fabaceae) for 18S rDNA, the *rbc*L sequence comparison yields 191 mutational differences (13.3% of the 1431 sites), whereas comparison of 18S sequences yields 138 differences (7.6% of the ca. 1810 sites). Similar comparisons using angiosperms show that *rbc*L is generally about three times more variable than 18S rDNA. For example, comparison of *Pisum* and *Spinacia rbc*L sequences demonstrates that 139 of the 1428 sites (9.7%) are different. In contrast, a similar comparison of *Glycine* (Fabaceae) and *Spinacia* 18S rDNA sequences indicates that only 62 of the ca. 1808 (3.4%) sites are different. Not only is the rate of evolution of *rbc*L considerably higher than that of the 18S gene (about 3 times faster), but even when the greater length of the 18S gene is taken into consideration, *rbc*L still exhibits approximately 1.4 times as many variable sites as 18S rDNA. The distribution of variable sites for the two molecules is also quite different. When the number of steps from one of the equally most parsimonious *rbc*L and 18S rDNA cladograms (e.g., Figs. 6 and 8, respectively) is plotted against site, the different variability patterns are graphically illustrated (Figs. 3 and 4). For *rbc*L, sites in general are more variable, and, although certain regions clearly are more variable than others, this variability appears more evenly distributed over the entire length of the molecule (Fig. 3) than for 18S (Fig. 4). That is, 18S rDNA shows highly variable regions interspersed with regions of extreme conservation (Fig. 4). Significantly, the variable domains indicated on the secondary structure of *Glycine* (V1–V9, Fig. 1) can be readily identified in Figure 4. The secondary structural study conducted by Senecoff & Meagher (1992) used dimethyl sulfate to modify (and thereby identify) adenine and cytosine residues of single-stranded portions of the soybean 18S rRNA molecule. Their data largely confirm the higher-order structure shown in Figure 1, especially for variable regions 1 and 4.

Pairwise 18S rDNA sequence comparisons within the flowering plants examined here indicated that most angiosperms differ from the above noted *Glycine* sequence at only 1–5% of the sites. Higher than average rates (numbers) of nucleotide substitution in 18S rDNA can be seen, however, in certain parasitic plants such as members of Viscaceae and Cuscutaceae (included in the present study) and Balanophoraceae, Hydnoraceae, and Rafflesiaceae (Nickrent & Starr, 1994). Representatives of the latter three families were not included herein because they apparently lack an *rbc*L gene (Nickrent & dePamphilis, unpublished data). The causes of such elevated rates of 18S sequence evolution are currently under investigation by the first author.

For both data sets, transitions outnumber transversions by approximately a factor of two. For the *rbc*L data set, there were 1520 unambiguous transitions and 862 unambiguous transversions; for 18S rDNA, there were 1099 and 574 unambiguous transitions and transversions, respectively. The specific types of mutational events for the two molecules are also very similar, differing mainly in the frequency of the A to G transition (14.6% of the total changes for *rbc*L, 5.3% of the total for 18S rDNA). Steps calculated over the *rbc*L tree by codon position demonstrate that most changes occur, as expected, in the third position followed by first and second position.

Phylogenetic analyses

rbcL. The heuristic search of the *rbc*L data matrix yielded 12 most parsimonious trees, all in one island, of length 3090 with a consistency index excluding uninformative substitutions (C.I.−) of 0.284 and retention index (R.I.) of 0.467. The strict consensus tree is illustrated in Figure 5. The main differences among the 12 most parsimonious trees

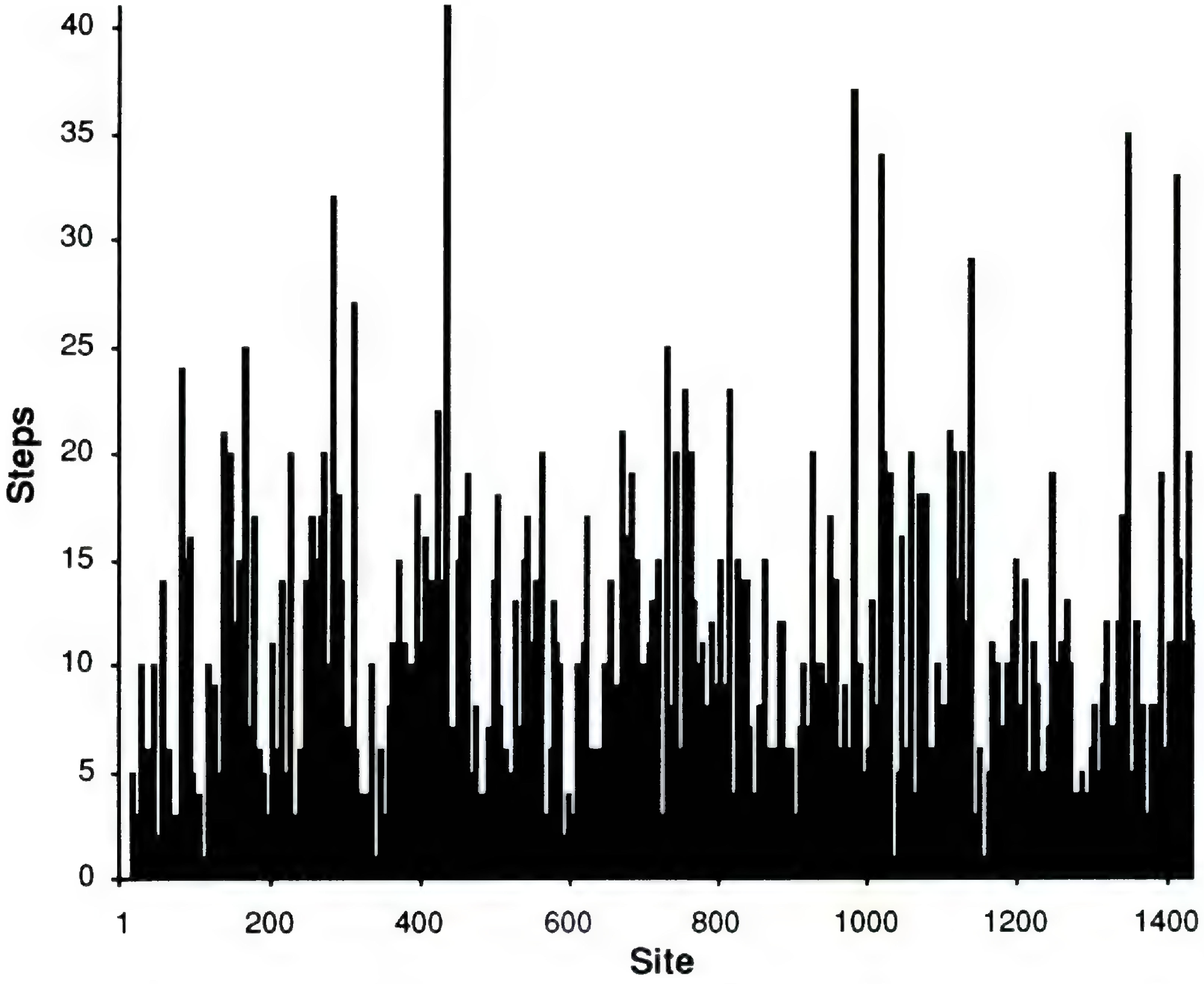


FIGURE 3. Variability histogram for *rbc*L. The number of steps (y axis) were determined from *rbc*L tree number 3 (of 12 equally parsimonious trees) as shown in Figure 6 with the interval widths (x axis) set to four base pairs. Variability is distributed relatively evenly over the 1431 sites of the molecule.

were the relationships among members of Santalales and in the relative position of *Lambertia* to nonpaleoherb dicots. The strict consensus tree shares a number of features with the *rbc*L reference tree (Fig. 2). Using *Zamia* as the outgroup, the two representatives of Gnetales (*Ephedra* and *Gnetum*) form a monophyletic group strongly supported by 66 synapomorphies that is sister to the angiosperms (Fig. 6). The angiosperms form a monophyletic group united by 39 base substitutions (bootstrap value of 93%). Within the angiosperms, the monocots examined (*Zea*, *Oryza*, and *Sparganium*), with the exception of *Acorus*, are the sister group to all other angiosperms. This relationship differs from the reference tree where the monocots, including *Acorus*, are monophyletic and are sister to the Magnoliales/Paleoherbs II group. In the present analysis, the Paleoherbs I group (*Houttuynia*, *Peperomia*, *Asarum*, *Saruma*, and *Aristolochia*) is disrupted by the inclusion of *Acorus* and *Drimys* (Magnoliales). The Ranunculids (*Akebia*, *Mahonia*, and *Ranunculus*) have the same composition and general topology as seen in the reference tree. This latter clade is part of a trichotomy in the strict consensus tree of Figure 5 that also comprises *Lambertia* (the single representative of the Hamamelid I group) and the remaining dicots. The present analysis of *rbc*L sequences does not include the closest relatives of *Lambertia* (i.e., *Sabia*, *Nelumbo*, *Platanus*) as determined in the Chase et al. analysis. *Pachysandra*, representing Hamamelid II, occupies a similar position on the strict consensus and reference trees. Several of the remaining clades have taxon compositions identical to those of the reference tree, although the topologies within these clades are not necessarily identical to those of the reference tree. These clades of identical composition include Asterid III (*Pyrola*, *Polemonium*, and *Impatiens*), Rosid II (*Brassica*, *Tropaeolum*,

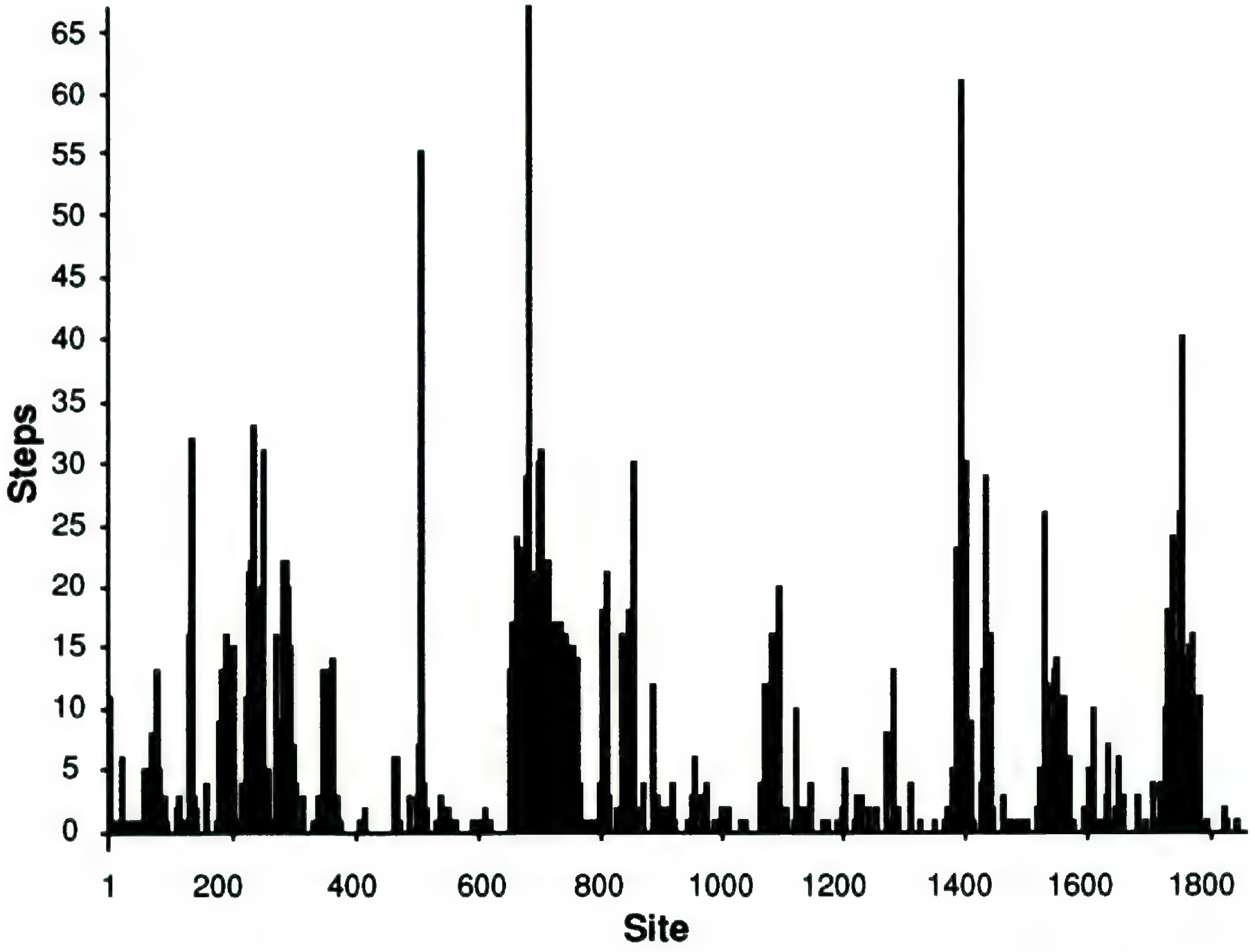


FIGURE 4. Variability histogram for 18S rDNA. The number of steps were determined from 18S rDNA tree number 5 (of 26 equally parsimonious trees) as shown in Figure 8 with the interval widths set to four base pairs. Regions of variability, generally concentrated in the variable domains (Fig. 1), are interspersed with extremely conserved sites over the 1853 total sites for the molecule.

and *Gossypium*), Santalales, Asterid II (*Pittosporum*, *Hedera*, and *Hydrocotyle*), and Asterid I (*Lycoperiscon* and *Convolvulus*). The members of Asterid IV (*Hydrangea*, *Cornus*, and *Nyssa*) do not form a monophyletic group herein, but do appear near each other at the base of the Asterid I and II groups. The sole representative of the Caryophyllids, *Spinacia*, was positioned within the Rosid I clade as opposed to sister to Santalales on the reference tree. The position of Caryophyllids differed also in the two searches conducted by Chase et al. (1993).

In our 62-taxa *rbc*L analysis, the Rosid I and Rosid III clades are nearly identical in composition to those of Chase et al. (1993). In our strict consensus tree, all taxa of the Rosid I clade, with the exception of *Francoa* and the addition of *Spinacia*, form a monophyletic group (compare Figs. 2 and 5). However, the omission of *Francoa* from this clade again likely reflects taxon density. The closest relatives of *Francoa* based on the Chase et al. (1993) analysis (*Greyia*, *Viviana*, *Wendtia*) were not included herein. With the exception of *Paeonia*, Rosid III also appears as a monophyletic group in our analysis of *rbc*L sequences. One should regard this difference with great caution given that the position of *Paeonia* shifts dramatically between the two searches of Chase et al. (1993). The San-

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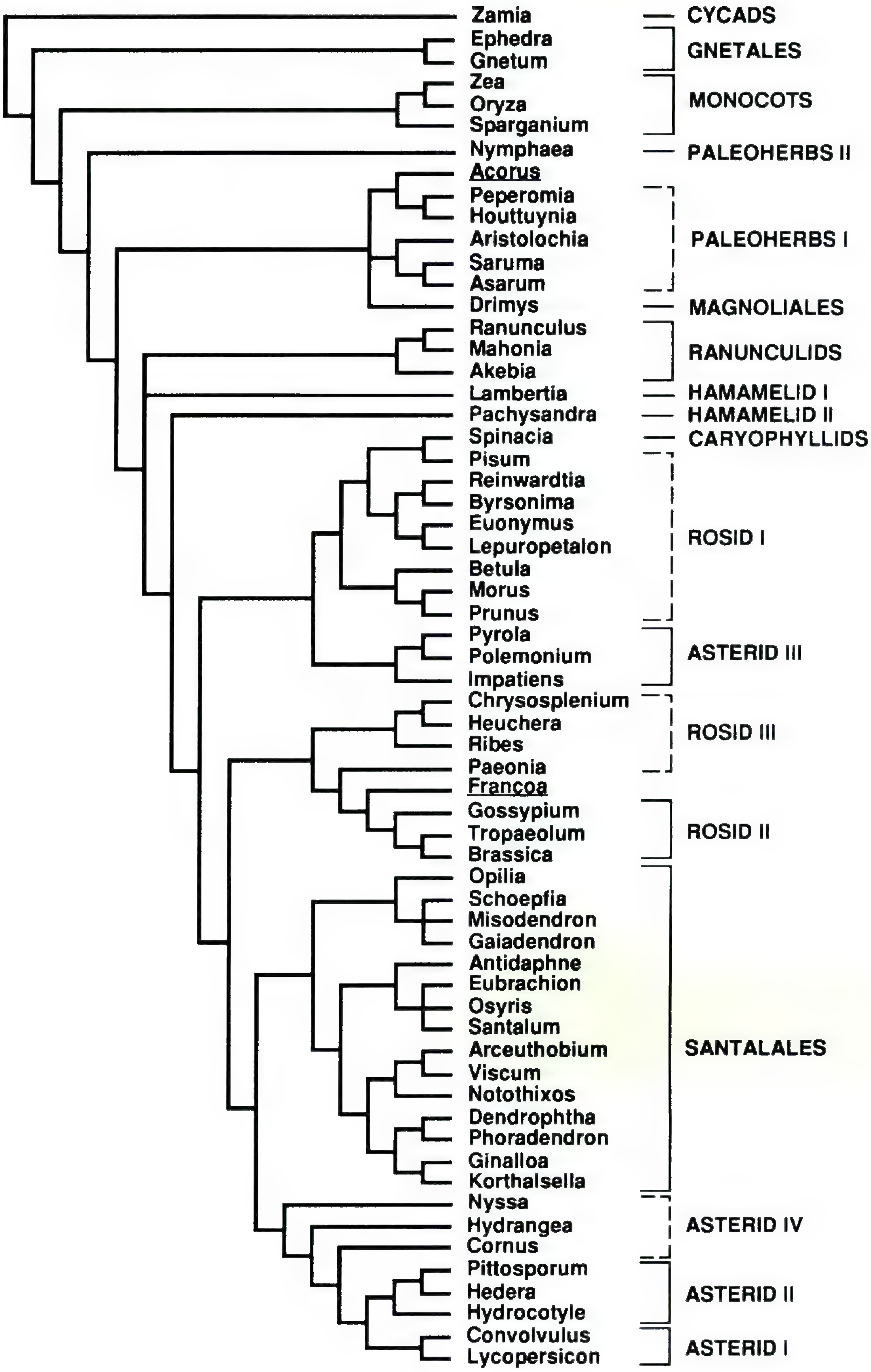
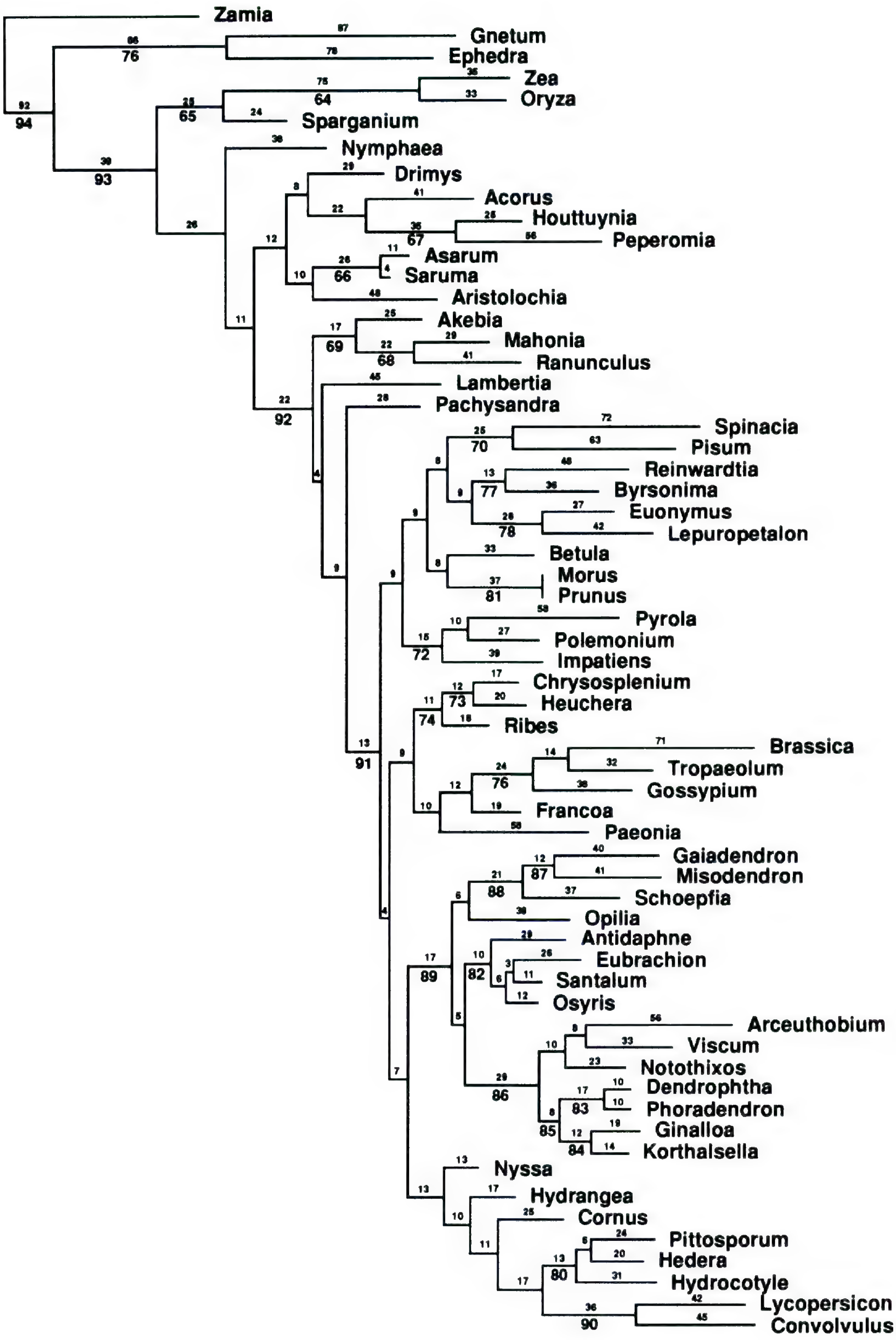
FIGURE 5. The strict consensus tree of 12 equally most parsimonious cladograms derived from a heuristic search of the 62-taxon *rbc*L matrix; tree length = 3104, C.I. minus uninformative sites = 0.283, R.I. = 0.463. Groups whose compositions are identical to those of the reference tree (Fig. 2) are indicated by solid braces. Groups that appear para- and polyphyletic (relative to the reference tree) are indicated by dashed braces. The positions of underlined taxa (*Acorus* and *Francoa*) differ significantly from those expected from the reference tree (monocots and Rosid I, respectively).


Zamia
Ephedra
Gnetum
Zea
Oryza
Sparganium
Nymphaea
Acorus
Peperomia
Houttuynia
Aristolochia
Saruma
Asarum
Drimys
Ranunculus
Mahonia
Akebia
Lambertia
Pachysandra
Spinacia
Pisum
Reinwardtia
Byrsonima
Euonymus
Lepuropetalon
Betula
Morus
Prunus
Pyrola
Polemonium
Impatiens
Chrysosplenium
Heuchera
Ribes
Paeonia
Françoa
Gossypium
Tropaeolum
Brassica
Opilia
Schoepfia
Misodendron
Gaiadendron
Antidaphne
Eubrachion
Osyris
Santalum
Arceuthobium
Viscum
Notothixos
Dendrophtha
Phoradendron
Ginalloa
Korthalsella
Nyssa
Hydrangea
Cornus
Pittosporum
Hedera
Hydrocotyle
Convolvulus
Lycopersicon
CYCADS
GNETALES
MONOCOTS
PALEOHERBS II
PALEOHERBS I
MAGNOLIALES
RANUNCULIDS
HAMAMELID I
HAMAMELID II
CARYOPHYLLIDS
ROSID I
ASTERID III
ROSID III
ROSID II
SANTALALES
ASTERID IV
ASTERID II
ASTERID I


Zamia
Gnetum
Ephedra
Zea
Oryza
Sparganium
Nymphaea
Drimys
Acorus
Houttuynia
Peperomia
Asarum
Saruma
Aristolochia
Akebia
Mahonia
Ranunculus
Lambertia
Pachysandra
Spinacia
Pisum
Reinwardtia
Byrsonima
Euonymus
Lepuropetalon
Betula
Morus
Prunus
Pyrola
Polemonium
Impatiens
Chrysosplenium
Heuchera
Ribes
Brassica
Tropaeolum
Gossypium
Francoa
Paeonia
Gaiadendron
Misodendron
Schoepfia
Opilia
Antidaphne
Eubrachion
Santalum
Osyris
Arceuthobium
Viscum
Notothixos
Dendrophtha
Phoradendron
Ginalloa
Korthalsella
Nyssa
Hydrangea
Cornus
Pittosporum
Hedera
Hydrocotyle
Lycopersicon
Convolvulus

talales form a monophyletic group in the present analysis, and their position here as sister to the Asterid clades is similar to the results of Search I of Chase et al., where the Santalales (represented in Chase et al. only by *Phoradendron*, *Schoepfia*, and *Osyris*), along with *Gunnera*, appear as the sister to all other asterids. In contrast, in Search II of Chase et al., the Santalales appear as sister to a clade containing the Caryophyllids. In the detailed analysis of *rbc*L sequences of asterids conducted by Olmstead et al. (1993), Santalales are not a component of Asteridae s.l. and they are likely members of a broadly defined rosid clade.

Bootstrap values and branch lengths of the 62-taxa *rbc*L tree (Fig. 6) suggest the presence of several strongly supported major clades within the angiosperms. The monocots, Paleoherbs, and Magnoliales (represented by *Drimys*) appear as the sister to the remainder of the angiosperms, which are supported as a monophyletic group by a high bootstrap value (92%). This large clade comprises most of the taxa included in the corresponding clade of Figure 2 and represents the "eudicots," which have triaperturate or triaperturate-derived pollen (Donoghue & Doyle, 1989; Chase et al., 1993; Qiu et al., 1993). Within this large eudicot clade, the Ranunculids, *Lambertia*, and *Pachysandra* appear as the sister to another large, strongly supported clade (bootstrap value of 91%). There are, however, few strongly supported subclades within this large clade. Subclades that received moderate to strong support (bootstraps of 70–80%) include the Asterid I, II, III, and IV, Rosid II, Rosid III minus *Paeonia*, and Santalales. Within the Santalales, the monophyly of the mistletoe family, Viscaceae, is supported by a bootstrap value of 86%.

18S-rDNA. Cladistic analysis of the 18S rDNA matrix yielded 26 equally parsimonious trees of length 2021, all in one island. Each of these trees had a C.I.− of 0.301 and an R.I. of 0.440. The strict consensus tree (Fig. 7) reveals that the Gnetales again appear as the sister to the angiosperms and that the angiosperms form a well-supported monophyletic group (bootstrap value of 100%, Fig. 8). Most clades in the 18S consensus tree are derived from a large polytomy, whereas the *rbc*L consensus tree (Fig. 5) displays considerable resolution. In the 18S analysis, the monocots *Zea*, *Oryza*, and *Sparganium* (minus *Acorus*) form a monophyletic group (bootstrap value of 91%) as does each of the following: Ranunculids, Rosid III, Santalales, Asterid I, and Asterid II.

A bootstrap analysis of the 18S rDNA data set (Fig. 8) indicates that *Acorus* and then *Nymphaea* are the sisters to a large clade containing the remaining angiosperms; however, these relationships received only weak support (bootstrap values less than 50%). A group of paleoherbs (*Asarum*, *Saruma*, *Aristolochia*, *Houttuynia*, and *Peperomia*) then appears as the sister to all remaining taxa. The large remaining clade corresponds, with one exception, to the "eudicots," as defined by Chase et al. (1993). The main discrepancy in the composition of eudicots between the *rbc*L tree and the 18S rDNA tree pertains to the relationships of *Drimys*: in contrast to the *rbc*L tree, the 18S tree places *Drimys* in the eudicot clade as sister to *Glycine*. The 18S eudicot clade is defined by only six base substitutions (Fig. 8) and is not present in the strict consensus tree (Fig. 7), whereas in the *rbc*L analysis, 22 base substitutions support this clade, and the bootstrap value is high (92%) (Fig. 6).

Despite the poorer resolution of the 18S than the *rbc*L tree, several subclades appear in both analyses. For example, the three genera of Aristolochiaceae (*Asarum*, *Saruma*, *Aristolochia*) form a monophyletic group, but the association with *Houttuynia* and *Peperomia*, the other subclade of the Paleoherb I group of Chase et al. (1993), is only weakly supported (bootstrap value less than 50%). The genera of Ranunculids (*Akebia*, *Mahonia*/*Podophyllum*, and *Ranunculus*) also form a monophyletic group in both trees, although the position of this clade is different.

Relationships within and among the several rosid clades show similarities in the 18S rDNA and *rbc*L trees, as well as several marked differences. 18S rDNA sequence data corroborate the results of *rbc*L sequence analysis in suggesting close relationships between some members of the Rosid I clade (Fig. 8, *Alnus* (representing Betulaceae), *Morus*, *Prunus*, *Francoa*, and *Malpighia* (representing Malpighiaceae)). In both the 18S and *rbc*L

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FIGURE 6. One of the 12 equally most parsimonious phylograms (cladograms that show branch lengths) derived from the heuristic search of the 62-taxon *rbc*L matrix; tree length 3090, C.I. minus uninformative sites = 0.284, R.I. = 0.467. Numbers above the branches indicate branch lengths (i.e., number of steps or nucleotide substitutions). Numbers below the branches indicate the percentage of trees (from 100 bootstrap replications) that support that node. Branches without bootstrap percentages were found in less than 50% of the trees.

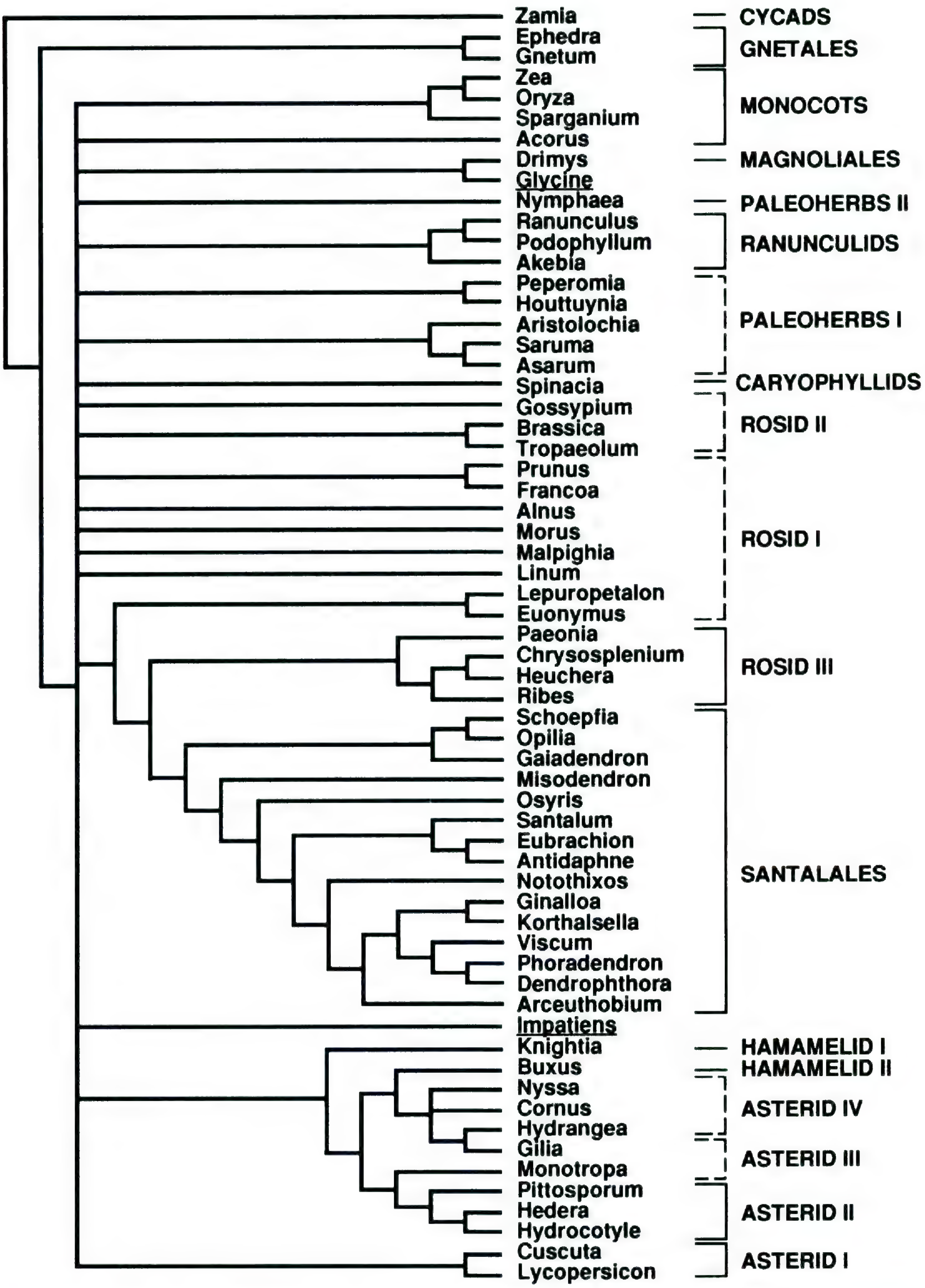

FIGURE 7. The strict consensus tree of 26 equally most parsimonious cladograms derived from a heuristic search of the 62-taxon 18S rDNA matrix; tree length = 2118, C.I. minus uninformative sites = 0.285, R.I. = 0.396. Groups whose compositions are identical to those of the reference tree (Fig. 2) are indicated by solid braces. Groups that appear para- and polyphyletic (relative to the reference tree) are indicated by dashed braces. The positions of underlined taxa (*Glycine* and *Impatiens*) differ significantly from those expected from the reference tree (Rosid I and Asterid III, respectively).

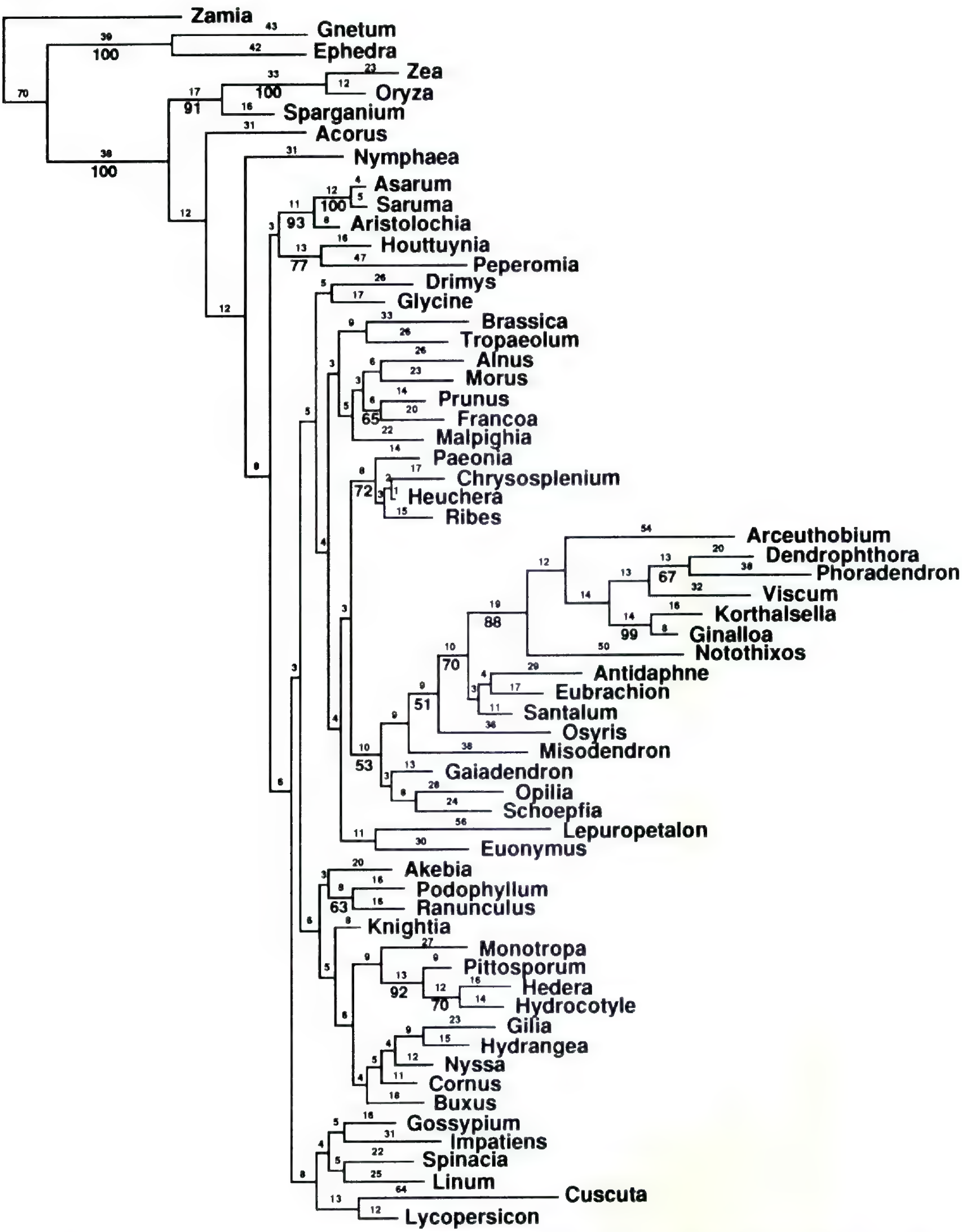

FIGURE 8. One of the 26 equally most parsimonious phylograms derived from the heuristic search of the 62-taxon 18S rDNA matrix; tree length 2021, C.I. minus uninformative sites = 0.301, R.I. = 0.440. Numbers above the branches indicate branch lengths (i.e., number of steps or nucleotide substitutions). Numbers below the branches indicate the percentage of trees (from 100 bootstrap replications) that support that node. Branches without bootstrap percentages were found in less than 50% of the trees.

sequence analysis, a close relationship is apparent between *Lepuropetalon* and *Euonymus*, and also *Morus* and *Betulaceae*. However, the position of Linaceae (represented by *Reinwardtia* in the *rbc*L analysis and by *Linum* in the 18S analysis) differs markedly between the 18S and *rbc*L trees. *Reinwardtia* is part of the Rosid I clade in the *rbc*L tree, whereas *Linum* is only distantly related to other Rosid I taxa in the 18S trees. Similarly, the placement of Fabaceae differs in the two analyses with *Pisum* appearing with other Rosid I taxa in the *rbc*L tree, but with *Glycine* appearing as the sister of *Drimys* in the 18S tree.

Both 18S and *rbc*L sequence data suggest a close relationship between *Brassica* and *Tropaeolum* (Rosid II; see Rodman et al., 1993). However, the placement of *Gossypium* differs markedly in the two trees. In the *rbc*L tree (Fig. 5), *Gossypium* is the sister of *Brassica* and *Tropaeolum*; all three genera are part of the Rosid II clade of Chase et al. (1993). In contrast, *Gossypium* is the sister of *Impatiens* in the 18S tree (Fig. 8) and is well removed phylogenetically from other Rosid II taxa.

Phylogenetic analyses of 18S and *rbc*L sequences also agree in suggesting a close relationship among *Chrysosplenium*, *Heuchera*, and *Ribes*, members of the Rosid III clade of Chase et al. (1993). The 18S analysis also places *Paeonia* in this clade, as does one of the two searches of Chase et al. (1993). Both analyses also concur in recognizing a well-supported monophyletic Santalales, although the position of this large clade differs between the two analyses. In the *rbc*L tree, Santalales appear as the sister to members of Asteridae sensu lato (Olmstead et al., 1993), whereas in the 18S analysis (Fig. 8) Santalales form the sister group of the Rosid III clade.

Several of the relationships among Asterid taxa seen in the *rbc*L analysis are also found in the shortest 18S trees. For example, *Lycopersicon* and Convolvulaceae (represented by *Convolvulus* and *Cuscuta* in the *rbc*L and 18S analyses, respectively) are sister taxa in both analyses. These taxa represent the Asterid I group of Chase et al. (1993). Similarly, the Asterid II group of *Pittosporum*, *Hedera*, and *Hydrocotyle* form a monophyletic group in both analyses. The Asterid IV subclade (Chase et al., 1993) that includes *Nyssa*, *Cornus*, and *Hydrangea* (along with *Gilia*, Polemoniaceae) also forms a subclade in the 18S analysis. In the *rbc*L consensus tree, these three genera do not form a monophyletic clade but are closely allied basal members of an Asterid assemblage.

In contrast to these similarities between the 18S and *rbc*L trees, the placement of those taxa representing the Asterid III clade (Chase et al., 1993) differs between the most parsimonious 18S and *rbc*L trees. In the 18S analysis (Fig. 8), Polemoniaceae (represented by *Gilia*) and *Monotropa* appear in clades with other Asterids. As previously mentioned, *Impatiens* emerges as sister to *Gossypium*. In contrast, *Polemonium*, *Pyrola* (a genus closely allied with *Monotropa*, Kron & Chase, 1993), and *Impatiens* form a subclade allied with the Rosid I clade in the shortest *rbc*L trees. This difference, however, may well reflect taxon density given that *Polemonium* and *Pyrola* are part of the Asteridae sensu lato when larger numbers of *rbc*L sequences are analyzed (Chase et al., 1993; Olmstead et al., 1993).

Several other placements and relationships differ dramatically between the 18S and *rbc*L trees. These include the phylogenetic positions of *Buxus*, *Gossypium*, *Spinacia*, and the sister-group relationship of *Drimys* and *Glycine* suggested by the 18S analysis. The same close relationship between *Drimys* and *Glycine* was also seen in the ribosomal RNA phylogenetic analysis of Hamby & Zimmer (1992).

Relationships within Santalales. As noted above, we analyzed Santalales in more detail to compare the resolution of 18S and *rbc*L sequence data at lower taxonomic levels. The *rbc*L sequence data reveal the presence of three clades within the order: (1) *Gaiadendron*, *Misodendron*, *Schoepfia*, *Opilia*; (2) *Antidaphne*, *Eubrachion*, *Osyris*, *Santalum*; and (3) *Arceuthobium*, *Dendrophthora*, *Phoradendron*, *Ginalloa*, *Korthalsella*, *Notothixos*, and *Viscum*. The first group, minus *Opilia*, is strongly supported (bootstrap value of 88%) as are the second (82%) and third groups (86%). Analysis of 18S sequences reveals a very similar pattern of relationship. The Viscaceae form a monophyletic group (88% bootstrap value). Considering group 2, *Antidaphne*, *Eubrachion*, and *Santalum* form a monophyletic clade, with *Osyris* as their sister. Lastly, *Gaiadendron*, *Opilia*, and *Schoepfia* form a subclade (minus *Misodendron*) that closely corresponds to the *rbc*L group 1.

Discussion

The goal of this project was not to resolve higher-level relationships among the angiosperms, but rather to evaluate the phylogenetic potential of complete 18S rDNA sequences through a comparison of molecular phylogenies derived from both *rbc*L and 18S rDNA using similar taxon sampling and identical density and familial representation. The enormous phylogenetic potential of *rbc*L se-

quences is now well documented by numerous studies. In contrast, the phylogenetic utility of entire plant small-subunit ribosomal RNA sequences may have been underestimated. The recent study of Hamby & Zimmer (1992) certainly suggested that partial 18S, as well as 26S, sequences might help resolve the deepest branches of angiosperm phylogeny. Nickrent & Franchina (1990) had previously demonstrated that complete sequences of the 18S region held considerable phylogenetic potential. The present study further illustrates the phylogenetic potential of entire 18S rDNA sequences.

The present study indicates clearly that the rate of evolution of 18S rDNA is lower than that of *rbc*L. The percentage of sites that are potentially phylogenetically informative is almost twice as high for *rbc*L as for 18S rDNA (33.6% vs. 18.4%). However, because the 18S region is almost 400 bp longer than *rbc*L, the ratio of the number of phylogenetically informative sites per molecule is only about 1.4 times greater for *rbc*L compared to 18S rDNA. Thus, the amount of variation afforded per molecule is more comparable than suggested by rate of evolution alone. Because the number of variable sites in 18S rDNA is lower than for *rbc*L, complete sequencing of the entire 18S region becomes more critical for phylogenetic inference. Not only does this approach maximize the number of variable sites, but complete sequencing concomitantly facilitates proper alignment of 18S sequences. The two molecules also differ greatly in terms of the distribution of variation along each respective DNA region (Figs. 3 and 4). That is, base substitutions are spread much more evenly across the entire length of *rbc*L than for 18S rDNA.

Considerable variation in the evolutionary rate of *rbc*L has been shown within the angiosperms (Wilson et al., 1990; Bousquet et al., 1992; Chase et al., 1993). Although lineage rate asymmetry can contribute to spurious branch attractions (Hendy & Penny, 1989; Albert et al., 1993), it may not be extensive enough between angiosperm lineages to be problematic in terms of phylogenetic reconstruction given sufficient taxon density (Chase et al., 1993). The extent of heterogeneity of evolutionary rates among most plant lineages for 18S rDNA is not yet known. Unequal rates of 18S rDNA sequence evolution are suggested, however, for some Santalales and other parasitic plants, which exhibit an accelerated rate of evolution compared to other angiosperms (Nickrent & Franchina, 1990; Nickrent & Starr, 1994). The number of nucleotide substitutions per site (K) in pairwise comparisons among five nonparasitic angiosperms averaged 0.036 for 18S rDNA (Nickrent & Starr, 1994). In contrast, pairwise comparisons using an obligate hemiparasite (*Arceuthobium*) and several holoparasites (*Prosopanche, Balanophora, Rafflesia,* and *Rhizanthes*) result in a mean K value of 0.115 (Nickrent & Starr, 1994). Investigations of other heterotrophic angiosperms such as *Pholisma* (Lennoaceae) and *Cuscuta* (Convolvulaceae) have revealed similarly high substitution rates (Nickrent & Colwell, 1994). Accelerated substitution rates may also be present in *Lepuropetalon* and *Peperomia* based on the very long branch lengths these taxa exhibit (56 and 46, respectively). These long branch lengths could, however, simply be an artifact of the low taxon density of this analysis. It is noteworthy that *Peperomia* and *Lepuropetalon* also have much longer branch lengths than do their sister taxa in the *rbc*L tree depicted herein (Fig. 6), but this was not the case in the larger analysis of Chase et al. (1993) in which closer relatives of these taxa were included. Regardless of the cause of the long branch lengths in *Peperomia* and *Lepuropetalon*, the phylogenetic position of these taxa is similar in both the 18S and *rbc*L trees shown herein.

To evaluate the phylogenetic potential of 18S rDNA sequences, it is also important to elucidate the impact of secondary structure of the 18S rRNA transcript on phylogenetic reconstruction. As reviewed recently (Dixon & Hillis, 1993), major questions remain regarding phylogenetic analysis of rRNA or rDNA data. These questions include: should loop bases (non-pairing bases) and stem bases (pairing bases) both be used in phylogenetic reconstruction and, if so, should bases from each class (stems and loops) be considered equally informative and independent? Wheeler & Honeycutt (1988) recommended that stem base nucleotides be eliminated from phylogenetic analyses, or weighted by one-half. In contrast, in a detailed analysis of 28S rRNA genes from vertebrates, Dixon & Hillis (1993) found that characters from both stems and loops contain phylogenetic information. In addition, they found that stem bases sustain a greater number of compensatory mutations than would be expected at random, but the number of such mutations was less than 40% of that expected under a hypothesis of perfect compensation to maintain secondary structure. Dixon and Hillis therefore suggested that the weighting of stem characters be reduced by no more than 20% relative to loop characters in phylogenetic analyses. In an analysis of 18S rRNA sequences from echinoderms, Smith (1989) similarly reported that paired nucleotides were phylogenetically informa-

tive. Although the methods are at present not fully developed, incorporation of information from rRNA secondary (and tertiary) structure in phylogeny reconstruction algorithms is taking place (Van de Peer et al., 1993). These issues will require more attention in future phylogenetic studies of plants that use rDNA.

Our comparison of a similar suite of 62 taxa for both *rbc*L and 18S rDNA sequences yielded phylogenetic trees with a number of similar features, although we emphasize again that these trees should not be viewed as rigorous phylogenetic hypotheses for angiosperms. Both analyses revealed a well-supported monophyletic Gnetales as sister to a monophyletic Magnoliophyta, a result not too surprising given the sampling of taxa used. Within the angiosperms, both analyses revealed a monophyletic group of monocots (*Zea, Oryza, Sparganium*) that did not include *Acorus* as sister to other angiosperms. The distinctiveness of *Acorus* within the monocots was recently emphasized by Duvall et al. (1993). In both analyses, *Nymphaea* occurred in a similar position as sister to all other dicots. Both 18S rDNA and *rbc*L analyses recognized several identical clades, including two groups of Paleoherbs (*Houttuynia, Peperomia*; and *Asarum, Saruma, Aristolochia*), Ranunculids (*Akebia*, Berberidaceae, *Ranunculus*), several groups of Rosids (*Brassica, Tropaeolum*; *Morus*, Betulaceae; *Lepuropetalon, Euonymus*; *Chrysosplenium, Heuchera, Ribes*), Santalales, and several groups of Asterids (*Pittosporum, Hedera, Hydrocotyle*; *Lycopersicon*, Convolvulaceae). On a broader scale, very similar patterns of relationship are suggested among many of the Rosids and Asterids. Furthermore, both analyses suggest the presence of a large eudicot clade. At a lower taxonomic level, nearly identical subclades were revealed within the Santalales by both 18S rDNA and *rbc*L sequences. The degree of resolution achieved within Santalales using 18S rDNA sequences may, in part, reflect the accelerated rate of evolution of this region in this group of plants (Nickrent & Franchina, 1990; Nickrent & Starr, 1994).

The fact that phylogenetic analysis of 18S rDNA sequences for 62 taxa reveals relationships within angiosperms very similar to those obtained for a similar suite of taxa using *rbc*L sequences strongly suggests that questions of higher-level phylogeny in the angiosperms, as well as in seed plants in general, can be addressed with entire 18S rDNA sequences. The differences between the 18S and *rbc*L trees compared herein could, in large part, reflect taxon density, and also the use of different genera to represent some families (e.g., Polemoniaceae, Buxaceae, Convolvulaceae). Furthermore, the differences between the phylogenetic relationships gleaned from *rbc*L and 18S rDNA data may derive from their being, respectively, plastid and nuclear-encoded gene trees, neither of which perfectly represents the true species tree. Our analyses reinforce the findings of others (e.g., Nickrent & Franchina, 1990; Martin & Dowd, 1991; Hamby & Zimmer, 1992; Conran & Dowd, 1993; Hoot et al., 1995, this issue) in suggesting that sequencing of the 18S rDNA region holds considerable phylogenetic potential.

Although comparative sequencing of the entire 18S rDNA region holds potential for inferring phylogeny, we stress that this nuclear region will almost certainly not elucidate familial and generic level relationships to the extent possible with *rbc*L sequences simply because of the slower rate of evolution and lower overall number of base substitutions of 18S rDNA compared to *rbc*L. Whereas comparative *rbc*L sequencing has been used to resolve relationships within some angiosperm and gymnosperm families, including Onagraceae (Conti et al., 1993), Rosaceae (Morgan et al., 1994), Saxifragaceae s.s. (Morgan & Soltis, 1993), Taxodiaceae (Brunsfeld et al., 1994), Cupressaceae (Gadek & Quin, 1993), and Ericaceae (Kron & Chase, 1993), similar resolution with 18S sequences seems unlikely. In some santalalean families such as Viscaceae, 18S rDNA sequences have resolved generic-level relationships in a fashion comparable to that achieved via comparative *rbc*L sequencing. However, it is likely that the ability to resolve subfamilial and generic relationships in Santalales with 18S sequence data was facilitated by the higher substitution rate for this region in these taxa. Nonetheless, these results for Santalales illustrate that, in some instances, 18S sequence variation can be useful within families. Concomitantly, these findings also indicate that the ability of 18S rDNA sequences to provide sufficient resolution within any particular order or family must be determined empirically, just as for *rbc*L.

This study suggests that comparative sequencing of the 18S region should prove most useful for addressing phylogenetic relationships at the family level and above. Our results parallel those of Hoot et al. (1995) who showed in an analysis of Lardizabalaceae and other ranunculids that 18S rDNA sequences are more conserved than the two chloroplast genes employed (*rbc*L and *atp*B), but were useful in resolving relationships above the level of family. 18S rDNA sequence variation may be particularly well suited for addressing deeper phylo-

genetic branches within the angiosperms and in seed plants in general. The present study certainly indicates that additional sequencing of the entire 18S rDNA region is justified to obtain a broad sampling of angiosperms and other seed plants for eventual comparison with *rbc*L-based tree topologies (e.g., Chase et al., 1993). At present, only approximately 150 complete angiosperm 18S rDNA sequences exist (compared to over 1500 *rbc*L sequences for angiosperms). Further 18S rDNA sequencing within the monocots, Magnoliidae, Caryophyllidae, Hamamelidae, and Dilleniidae (sensu Cronquist) is especially needed to achieve greater taxon density for the angiosperms.

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CRYPTIC SEQUENCE SIMPLICITY, NUCLEOTIDE COMPOSITION BIAS, AND MOLECULAR COEVOLUTION IN THE LARGE SUBUNIT OF RIBOSOMAL DNA IN PLANTS: IMPLICATIONS FOR PHYLOGENETIC ANALYSES[1]

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Abstract

Sequences of the large subunit (LSU) of ribosomal DNA from *Arabidopsis thaliana, Brassica napus, Sinapsis alba, Oryza sativa, Fragaria ×ananassa, Lycopersicon esculentum,* and *Citrus limon* were analyzed for nucleotide composition, presence of "cryptic" sequence simplicity, and evidence of molecular coevolution among the 12 expansion segments. The median value for GC content across the seven plants for the LSU was 56%, but the distribution of GC was nonrandom. Expansion segments were decidedly more GC rich (65% on average) than were the conserved core regions (52% on average). Only *Oryza sativa* had significant cryptic sequence simplicity, which was found to be greatest in expansion segments D8 and D12. Sequence similarity between expansion segments also was strongest in rice as determined by visual inspection of dot plots. The complex nature of sequence variation in the LSU of rDNA complicates the use of this molecule as a molecular systematic marker.

Choosing an appropriate gene(s) is one of the most important steps for any molecular systematics study. The nature of sequence variation in a given gene influences all subsequent steps in the analysis, including the quality of the multiple sequence alignment (which serves as the basis for hypotheses of positional homology for each nucleotide or amino acid), and the outcome of the algorithm used for phylogenetic reconstruction. Friedlander et al. (1992) described several criteria for selecting "ideal" molecular systematic markers, including (1) genes that are present in single copies (or if present in multiple copies can be differentiated one from another or are homogeneous within a species), (2) genes or regions that are longer than 500 base pairs, (3) genes that contain both conservative and variable regions, (4) genes that lack significant nucleotide composition bias, and (5) genes that lack many or long introns. In addition to these structural/compositional factors, it also is desirable to know something of the modes of sequence variation patterns across a molecule to assess the potential for non-independence of characters and other violations of the assumptions which are made by different algorithms used for phylogenetic reconstruction.

The molecular biology, evolution, and general biosystematic utility of rDNA have been reviewed extensively and will not be addressed in detail here (Arnheim, 1983; Dover & Flavell, 1984; Gerbi, 1985; Appels & Honeycutt, 1986; Flavell, 1986; Schaal & Learn, 1988; Jorgensen & Cluster, 1988; Knaack et al., 1990; Larson, 1991; Hillis & Dixon, 1991). For systematic analyses, the small subunit of rDNA has been used most often, especially for deep evolutionary divergences (e.g., Zimmer et al., 1989; Nickrent & Soltis, 1995, this issue). Recently, the internal transcribed spacer (ITS) regions that flank the 5S coding region in the rDNA repeat have gained attention as suitable markers for inter- and intra-generic comparisons in plants (e.g., Baldwin, 1992; Baldwin et al., 1995, this issue). The LSU has been used to a limited extent in plant systematics and all of the published nucleotide se-

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TABLE 1. Complete nuclear LSU rDNA sequences for plants in GenBank.

Taxon	Common name	GenBank accession	Reference
Arabidopsis thaliana	mouse-ear cress	X52320	Unfried & Gruendler, 1990
Brassica napus	rapeseed	D10840	Okumura & Shimada, 1992
Sinapsis alba	mustard	X57137	unpublished
Citrus limon	citrus	X05910	Kolosha & Fodor, 1990
Fragaria ×ananassa	strawberry	X58118	unpublished
Lycopersicon esculentum	tomato	X13557	Kiss et al., 1989
Oryza sativa	rice	M11585	Sugiura et al., 1985

quence-based analyses to date have used partial LSU sequence data (Hamby & Zimmer, 1988; Kantz et al., 1990; Zechman et al., 1990; Bult & Zimmer, 1993).

The rDNA LSU is a mosaic consisting of cores of highly conserved regions interspersed with 12 regions of variable size called "expansion segments" (Clark et al., 1984) or "divergent domains" (Hassouna et al., 1984). The number and relative positions of the expansion segments within the LSU are highly conserved among a wide range of taxa. Most of the fluctuations in length associated with expansion segments reflect the gain and loss of short, directly repetitive sequence motifs by a slippage-like mechanism during turnover (Hancock & Dover, 1988). Tautz et al. (1986, 1988) coined the term "cryptic sequence simplicity" to describe the sequence footprints of the motifs after they are shuffled among themselves by repeated slippage events. Unlike tandem arrays of unshuffled direct repeats (pure simplicity), cryptic simplicity is not easy to detect by simple visual inspection of a sequence. The elevated rates of sequence variation observed in expansion segments suggest that these regions lack function and, therefore, tolerate relatively high rates of point mutations and structural changes (Gerbi, 1985; Hancock & Dover, 1988; Tautz et al., 1988). However, the following three lines of evidence suggest that expansion segments do play a functional role, or at least evolve under selective constraints: (i) transcripts of the expansion segments are present in the mature 28S rRNA of some eukaryotes (Hassouna et al., 1984), (ii) dot plot comparisons of the complete LSU sequence from *Drosophila* demonstrate that expansion segments coevolve (Hancock & Dover, 1988), and (iii) expansion segments show a high degree of secondary structure conservation (Hancock et al., 1988). One hypothesis for the observed patterns of sequence change among and within the expansion segments is that they play some role in maintaining the steric integrity of the ribosome (Gerbi, 1985).

The complex mode of sequence variation observed in the LSU potentially complicates the use of this molecule as a phylogenetic marker. Motif shuffling and molecular coevolution among expansion segments will violate assumptions of character independence and compromise hypotheses of homology at many nucleotide positions in a multiple-sequence alignment. The purpose of the present study is to report on the nature of sequence variation across the large subunit (LSU) of ribosomal DNA (rDNA) in plants and discuss the implications of the observed patterns of variation for using the LSU as a molecular marker in phylogenetic analyses. We compare the conserved core and expansion segment region for full-length sequences of the large subunit of rDNA in plants to (1) assess nucleotide composition, (2) test for cryptic sequence simplicity, and (3) look for evidence of molecular coevolution among expansion segments.

MATERIALS AND METHODS

FULL-LENGTH LSU SEQUENCES FROM GENBANK

Seven full-length rDNA LSU sequences were found in version 79 of GenBank. The names and database accession numbers of these sequences are given in Table 1. Many more partial LSU sequence fragments are available in GenBank, but were not included in this analysis.

SEQUENCE SIMPLICITY

Sequence simplicity was analyzed using the SIMPLE34 program (Tautz et al., 1986; Hancock & Armstrong, 1994) running on a Sun SPARCstation 2 computer. The SIMPLE34 program is available via anonymous ftp or gopher from life.anu.edu.au or by sending an electronic mail message to John.Hancock@anu.edu.au. Sequence simplicity is evaluated by counting the number of tri- and tetranucleotides along the length of a DNA sequence using a 32-bp sliding window. The minimum length of a sequence that can be analyzed

by this method is 68 bp (Hancock & Armstrong, 1994). The statistical significance of the trimer and tetramer count is determined by comparing the results for the test sequence to values obtained for a set of ten randomly shuffled sequences (10,000 nucleotides long) having the same base composition as the test sequence. The score comparing the test sequence to the random sequence is called a Relative Simplicity Factor (RSF). An RSF value greater than 1 for a test sequence is statistically significant if the raw simplicity factor score for the test sequence is greater than 3 standard deviations of the simplicity factor value for the ten randomized sequences. RSF values significantly greater than 1 have cryptically simple direct repeats; RSF values significantly less than 1 have cryptically simple inverted repeats.

An underlying bias in dinucleotide composition for a particular sequence could, in turn, bias the statistical assessment of trimer and tetramer motifs in the RSF analysis. To correct for underlying dinucleotide composition bias, sequences that gave significant RSF scores were re-analyzed using a 2nd order Markov rule (Hancock & Armstrong, 1994).

DOT PLOT ANALYSIS

Dot plot comparisons were performed using the ABI Inherit Analysis software (v. 1.1; Applied Biosystems, Inc.) on a MacIntosh Quadra 900 computer to examine levels of sequence similarity between expansion segments (internal sequence similarity). The LSU of *Escherichia coli* (Brosius et al., 1980; GenBank Accession V00331) was included as a negative control as it contains no expansion segments (Clark et al., 1984; Hancock & Dover, 1988); the LSU of *Homo sapiens* (Gonzalez et al., 1985; GenBank Accession M11167) was included for comparison because it shows strong patterns of expansion segment similarities (Hancock & Dover, 1988). Internal sequence similarity was scored as present or absent by visual inspection of the dot plots. Dot plots were generated using a sliding window of 9 bp (with an offset of 3 bp) and an error tolerance of 33%. These parameters gave the best signal-to-noise ratio for detecting evidence of internal sequence similarity in plants. Because the human sequence has stronger patterns of internal sequence similarity than that observed for plants, the window was expanded to 10 bp (offset of 3; error tolerance of 20%) to decrease the background. Sequence simplicity profiles generated by the SIMPLE34 program were plotted along the X and Y axes of each dot plot to aid in visual interpretation. The profiles are graphical representations of levels of sequence simplicity over the length of the test sequence, averaged over blocks of 10 nucleotides.

TERMINOLOGY

We use the term expansion segment instead of divergent domain in this report because of the multiple meanings of the word *domain* in molecular biology. However, we retain the standard naming scheme of D1 through D12 to refer to individual expansion segments.

RESULTS AND DISCUSSION

NUCLEOTIDE COMPOSITION

The average GC content of plant LSU sequences is 56% (Table 2). The distribution of GC is not, however, randomly distributed throughout the length of the sequence. For all seven species examined, expansion segments have a higher average GC content (65%) than do the conserved core regions (52%).

SEQUENCE SIMPLICITY

Of the seven plant species analyzed, only rice (*Oryza sativa*) has statistically significant sequence simplicity (Table 2). The significance is retained even after a 2nd order Markov correction for underlying dinucleotide composition bias (data not shown). Although their overall RSF values are not significant, three of the taxa examined, *Arabidopsis*, *Sinapsis*, and *Fragaria*, show over-representation of a particular tetramer motif (CGGC, CGGA, and AAAG, respectively; data not shown). Analysis of the 12 expansion segments individually (Table 3) reveals that much of the overall simplicity in plants is concentrated in expansion segments D8 and D12. The RSF values for D8 are significant for *Arabidopsis*, *Citrus*, *Fragaria*, *Lycopersicon*, and *Oryza*; the RSF values for D12 are significant for *Arabidopsis*, *Citrus*, and *Oryza*. After correcting for underlying dinucleotide composition bias, only the RSF values for *Oryza* remain statistically significant. In some vertebrates (human, mouse, rat), RSF values are greatest in expansion segments D2, D6, D8, and D12 (Hancock & Dover, 1988).

We also applied sequence simplicity analysis to published full-length sequences of the LSU from plant chloroplast and mitochondrial genomes and to nuclear small subunit (16/18S, SSU) rDNA. The RSF values for these sequences are not statistically significant (data not shown).

TABLE 2. Nucleotide composition, relative simplicity factor (RSF) values, and internal sequence similarity for full-length sequences of LSU rDNA in plants. *Homo sapiens* and *Escherichia coli* are included for comparison (see text).

Taxon	Length (bp)	% GC* ALL	% GC* CORE	% GC* ES	RSF	Internal sequence similarity‡
Arabidopsis thaliana	3375	55	52	63	1.031	+/−
Brassica napus	3378	54	51	61	0.984	−
Sinapsis alba	3381	55	52	61	1.015	−
Citrus limon	3393	57	53	68	1.080	+
Fragaria ×*ananassa*	3377	56	52	66	1.039	+/−
Lycopersicon esculentum	3381	57	52	66	1.063	−
Oryza sativa	3377	59	53	71	1.184†	+
Homo sapiens	5025	69	nd	nd	1.681†	++
Escherichia coli	2904	53	nd	nd	1.015	−

* ALL = full-length sequence; CORE = conserved core regions; ES = expansion segments; nd = not determined.
† Denotes statistically significant RSF.
‡ Absence (−) or presence (+) of internal sequence similarity is determined by visual inspection of dot plots (Fig. 2).

The positions of the 12 expansion segments (D1–D12) relative to the sequence simplicity profile peaks for the LSU rDNA of rice are shown in Figure 1. The expansion segment coordinates of rice (unpublished data provided by John Hancock and Gabriel Dover) were mapped to a multiple-sequence alignment of the LSU rDNA from all seven plant species to determine position and length variation in the expansion segments and the conserved core regions. Relative positions of expansion segments and the core regions are conserved across all of the plant species analyzed. Some length variation was observed between species for the expansion segments and the conserved core regions. Most of the length variation in the conserved core regions was due to single position insertion/deletion events. The largest insertion/deletion event observed in any conserved core was a single gap involving 4 contiguous positions in the alignment. In contrast, most of the length variation in the expansion segments was due to insertion/deletion events involving at least 2 contiguous positions in the multiple-sequence alignment. The largest insertion/deletion event was observed in expansion segment D2 and

TABLE 3. Relative simplicity factor values for expansion segments (longer than 68-bp) of plant LSU of rDNA. Nucleotide composition (in %GC) given in parentheses.

Taxon	D1 [150]	D2 [224]	D3 [107]	D4 [8]	D5 [39]	D6 [27]	D7a [41]	D7b [27]	D8 [141]	D9 [24]	D10 [75]	D11 [4]	D12 [130]
	Expansion segment [median size in bp]												
Arabidopsis thaliana	0.716 (62)	0.958 (68)	0.803 (59)	—	—	—	—	—	1.464* (73)	—	0.404 (66)	—	1.270* (61)
Brassica napus	0.663 (62)	0.981 (66)	0.607 (61)	—	—	—	—	—	0.800 (66)	—	0.845 (62)	—	0.963 (61)
Sinapsis alba	0.730 (62)	0.992 (68)	0.705 (56)	—	—	—	—	—	1.127 (68)	—	0.845 (62)	—	0.855 (61)
Citrus limon	1.028 (70)	1.229 (75)	0.887 (61)	—	—	—	—	—	1.105* (76)	—	0.055 (69)	—	1.280* (72)
Fragaria ×*ananassa*	0.685 (66)	1.051 (72)	0.859 (56)	—	—	—	—	—	1.063* (72)	—	0.130 (64)	—	1.178 (66)
Lycopersicon esculentum	0.912 (68)	0.964 (73)	1.010 (58)	—	—	—	—	—	1.049* (75)	—	0.150 (64)	—	1.081 (67)
Oryza sativa	0.587 (71)	1.032 (79)	1.017 (64)	—	—	—	—	—	1.473† (82)	—	0.443 (68)	—	1.685† (80)

* RSF is not statistically significant after Markov correction for dinucleotide composition.
† Statistically significant RSF.

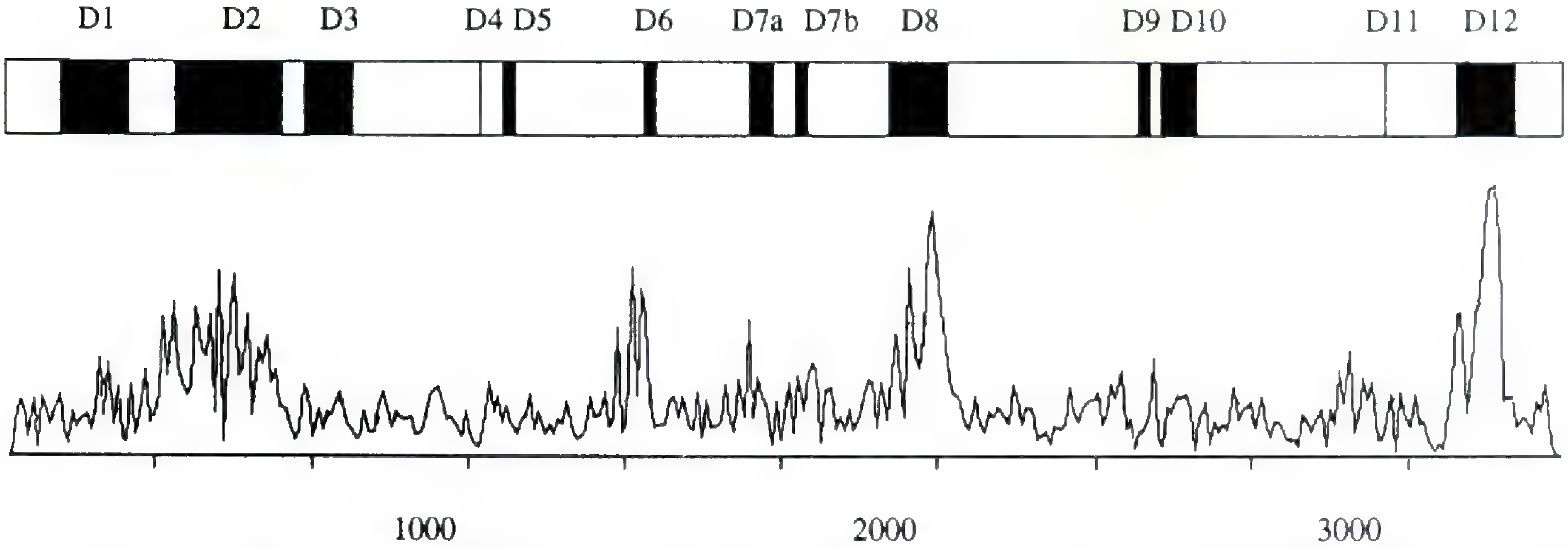


FIGURE 1. Locations of divergent domains (D1–D12) in the large subunit rDNA of *Oryza sativa* mapped to regions of sequence simplicity measured by SIMPLE34 (Hancock & Armstrong, 1994). Length of sequence given in base pairs.

involved 10 contiguous nucleotide positions in the alignment.

Overall sequence variation was much greater in the expansion segments than in the conserved core regions. Of 1034 nucleotide positions comprising the expansion segments, 431 (42%) were variable. Seventy of the 431 variable positions were due to insertion/deletion events. Of the 2423 nucleotides in the conserved core regions, 239 (10%) were variable. Forty-eight of the 239 variable positions in the conserved core regions were due to insertion/deletion events.

DOT PLOT ANALYSIS

A visual comparison of the dot plots of the LSU in plants to those for humans and bacteria reveals that the levels of internal sequence similarity are generally low in plants (Fig. 2). *Oryza* shows the strongest patterns of inter-expansion segment similarity, which appear as intensely dark regions on the dot plot. These regions of high similarity correspond well to the peaks of sequence simplicity associated with the expansion segments in the sequence simplicity profiles that border the dot plot. Interestingly, the dot plot of *Citrus limon* also shows a relatively strong pattern of expansion segment similarity. Because the RSF values for *Citrus* are not statistically significant, the similarity is most likely due to similar nucleotide composition in the expansion segment regions. This result highlights the importance of interpreting dot plot analyses in conjunction with sequence simplicity analyses.

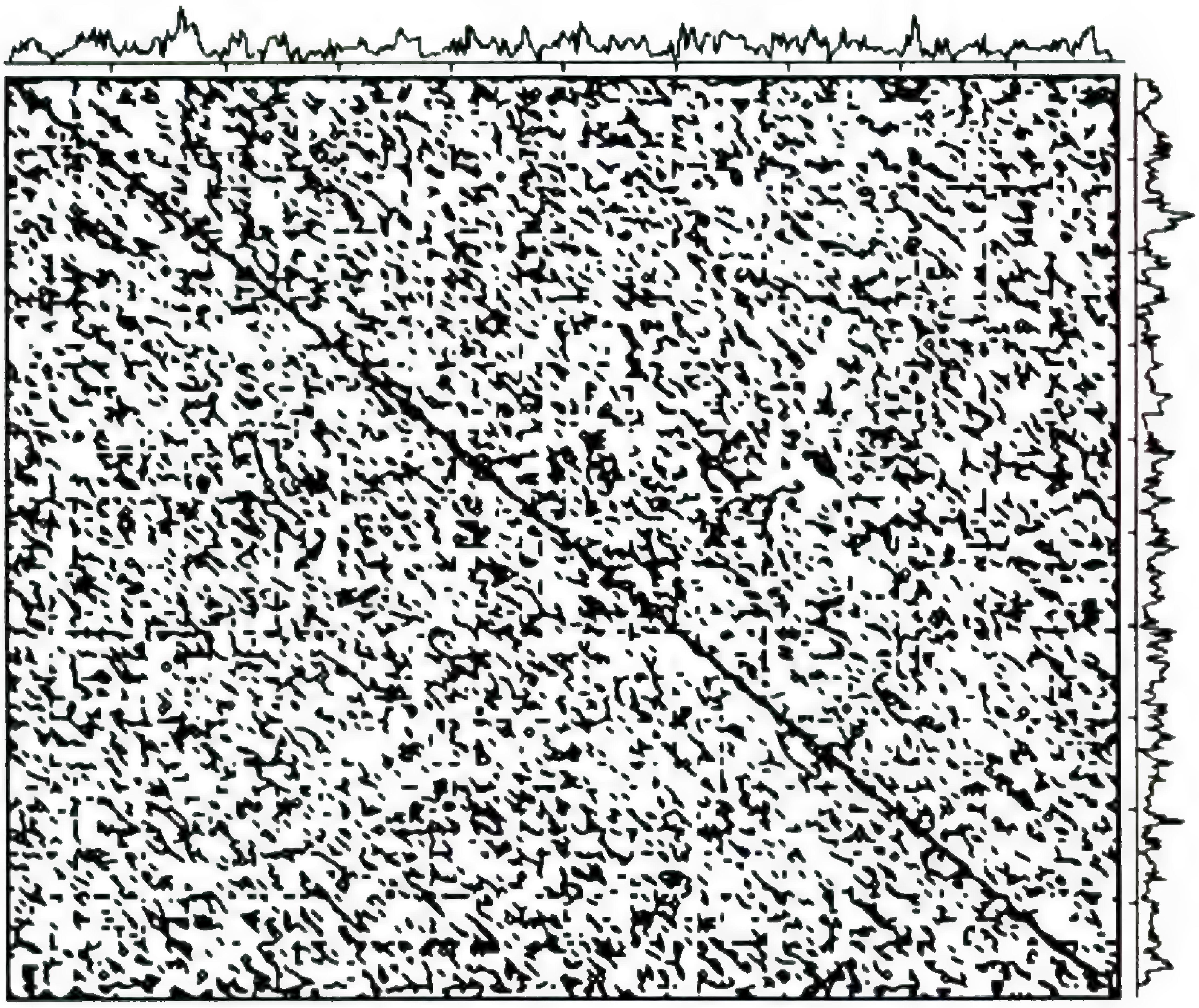
SUMMARY

The LSU of rDNA is often touted, anecdotally and in the literature, as an alternative gene to the small subunit (16/18S or SSU) of rDNA for phylogenetic analysis because it is longer and has a higher overall rate of sequence variability and, therefore, may provide more phylogenetically informative characters than the SSU for certain systematic questions (Larson, 1991). As demonstrated in this paper, and in the others cited herein, the patterns of nucleotide sequence change in the LSU are more complicated than is indicated by this "face value" assessment. Although the expansion segment regions of the LSU are potentially problematic as molecular markers in phylogenetic analyses, the conserved core regions may be useful as a source of molecular characters for exploring deep evolutionary divergences in the plant kingdom.

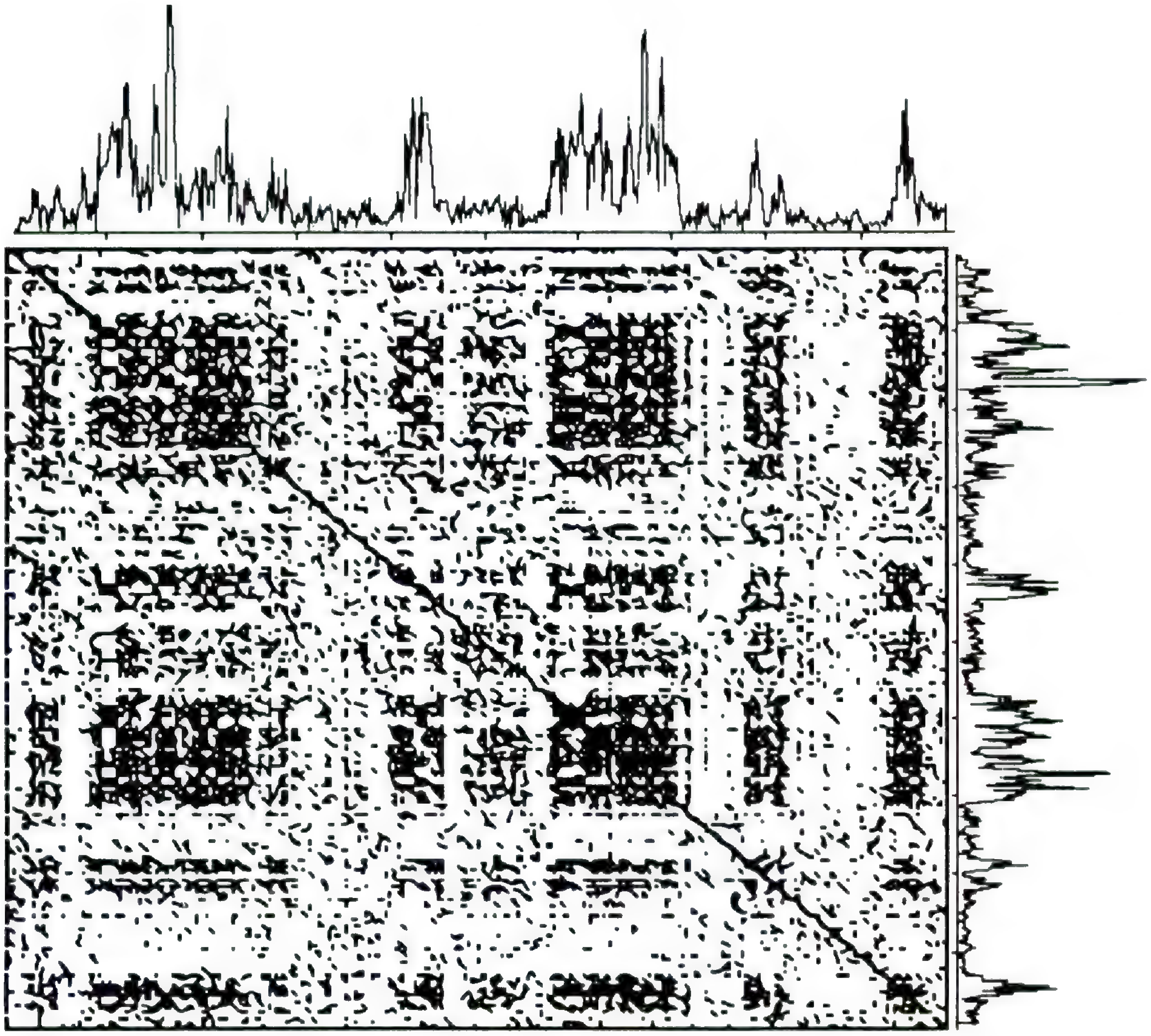
The analysis of the LSU for plants reveals the following: (1) nonrandom distribution and bias in nucleotide composition in expansion segments of all plant taxa examined, (2) significant cryptic sequence simplicity for *Oryza* and borderline simplicity for *Arabidopsis*, *Fragaria*, and *Sinapsis*, and (3) similarity among expansion segments (in *Oryza*) beyond that due to shared nucleotide composition. Nucleotide bias will complicate estimates of divergence times and will result in high levels of homoplasy due to convergence. For example, interspecific dot plot analyses by Hancock & Dover (1988, 1990) revealed a high degree of sequence similarity between human and rice due to nucle-

→

FIGURE 2A–I (pp. 240–244). Intra-specific dot plots of the complete sequences of LSU rDNA for seven plant species. Sequence simplicity profiles generated by SIMPLE34 border each dot plot. The higher the peak, the greater the level of sequence simplicity. Peaks of sequence simplicity generally correspond to expansion segments in eukaryotes. Dot plots for *Homo sapiens* and *Escherichia coli* are shown for comparison (see text).

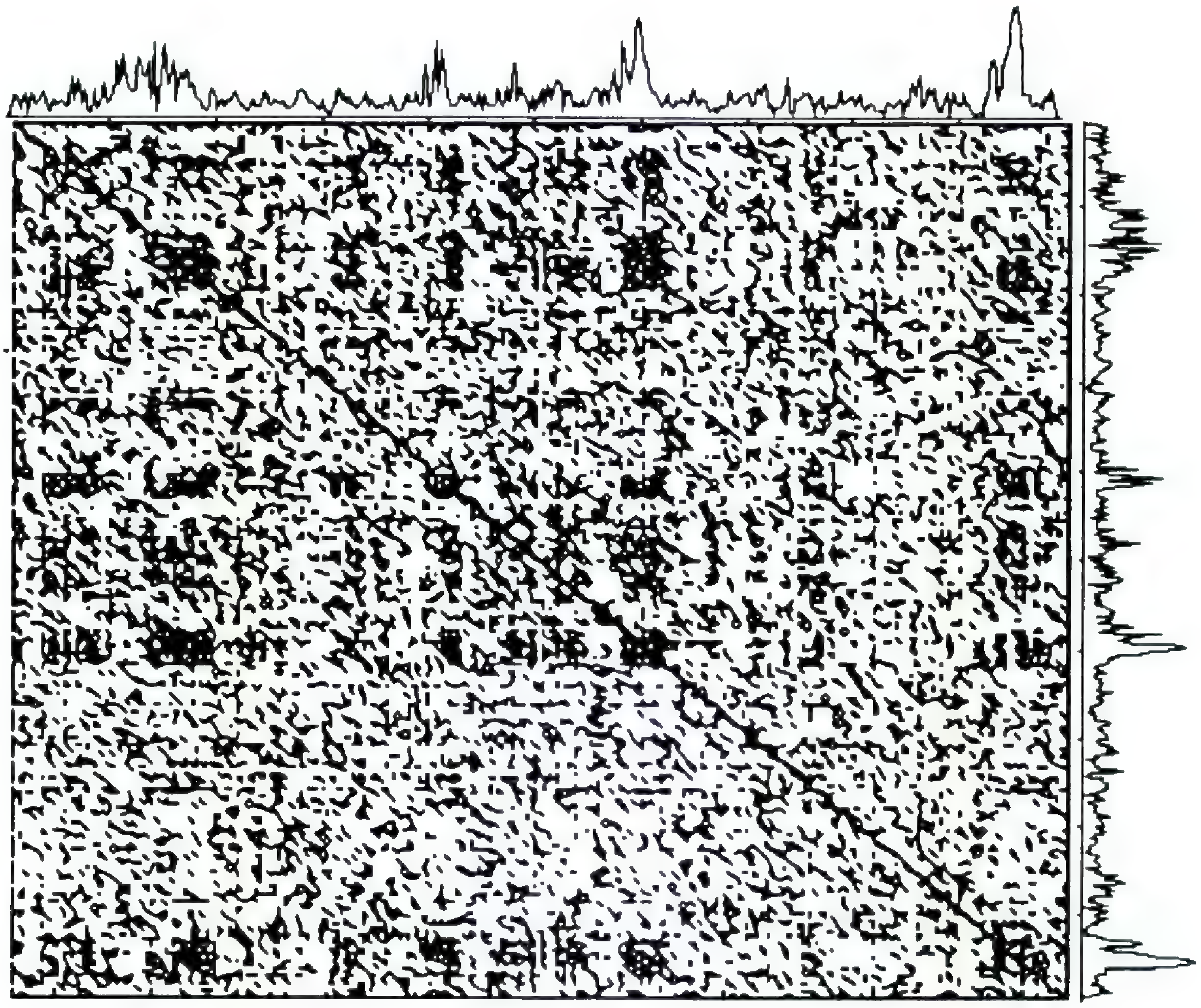


A. *Escherichia coli*

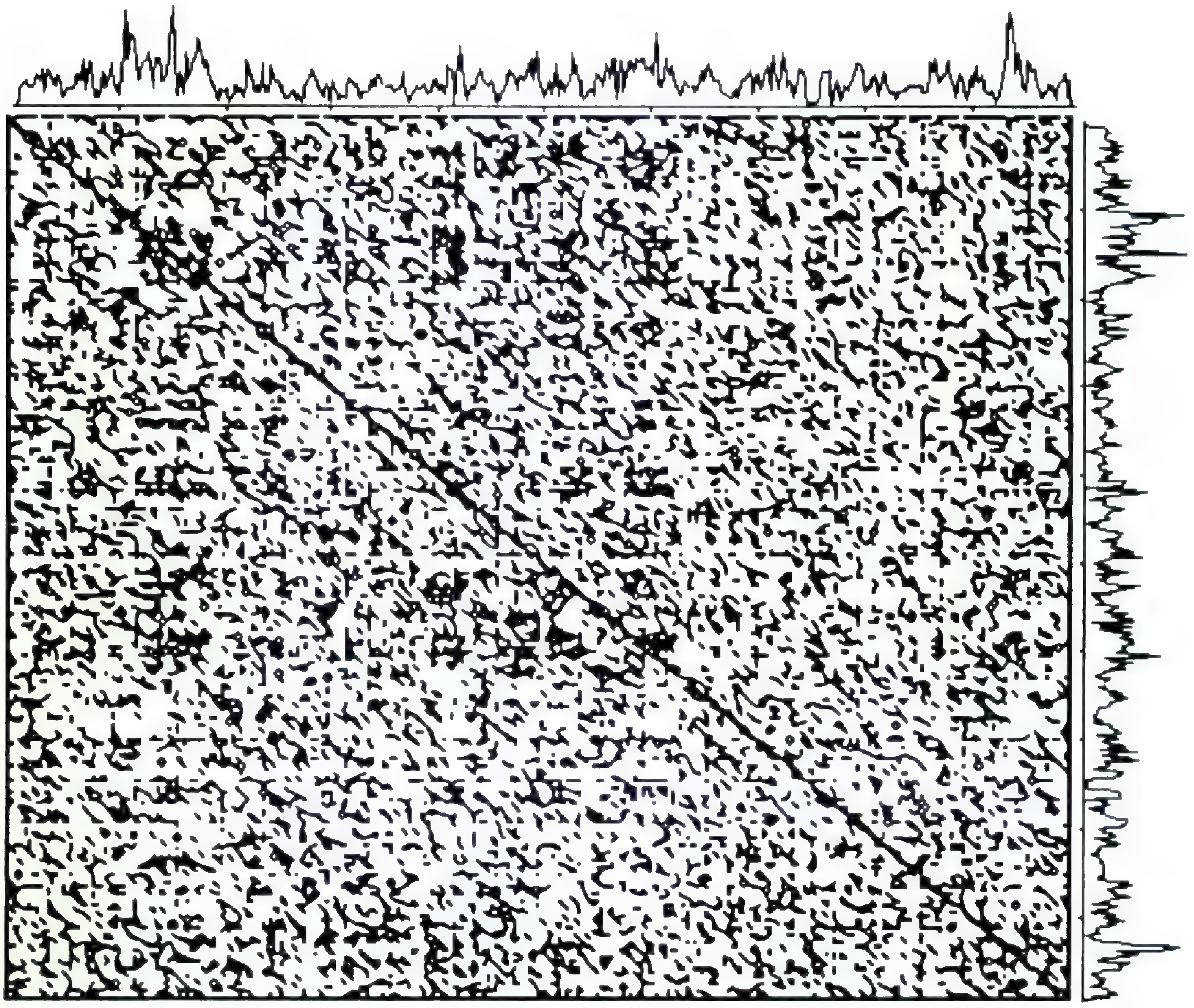


B. *Homo sapiens*

FIGURE 2. Continued.

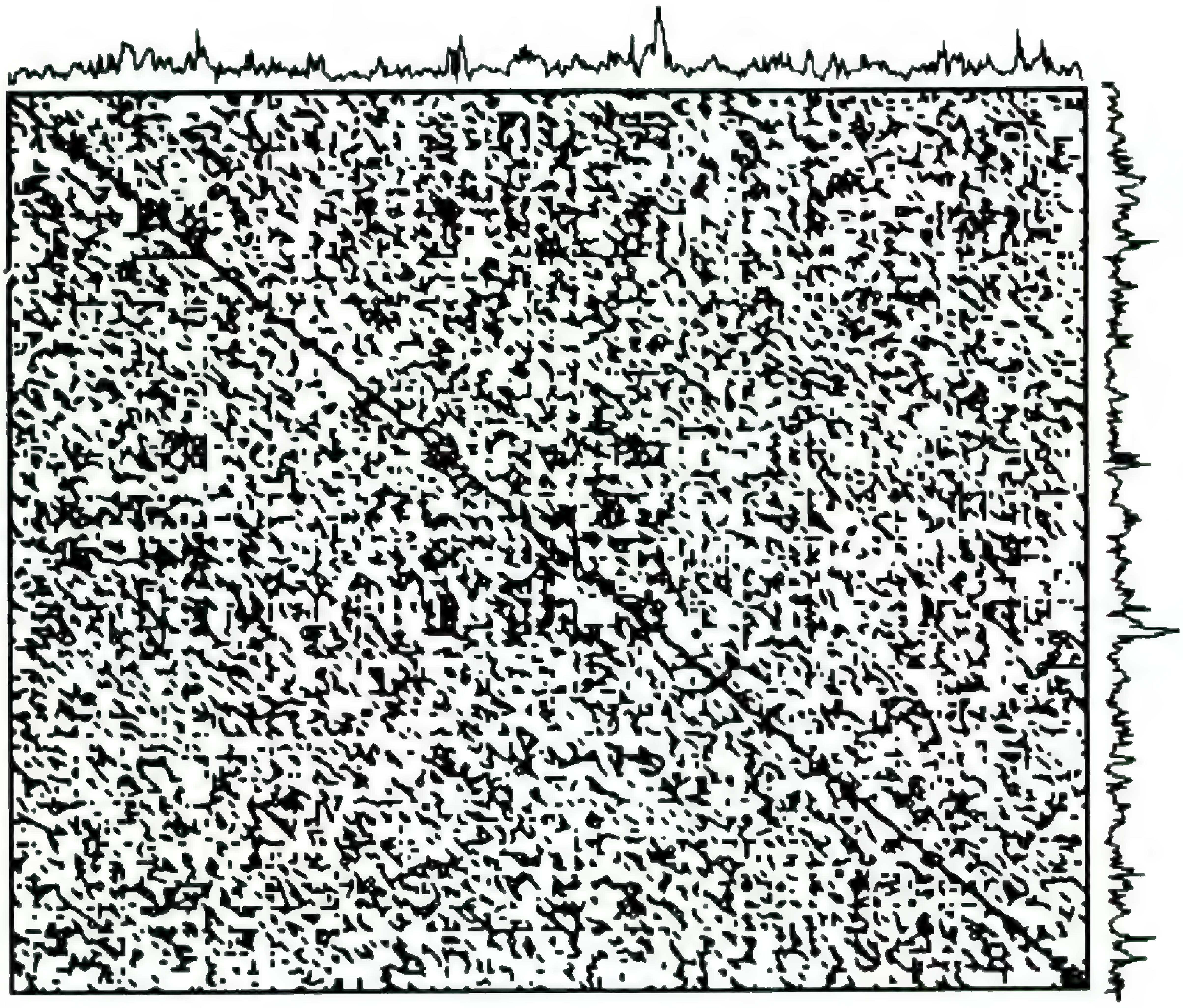


C. *Oryza sativa*

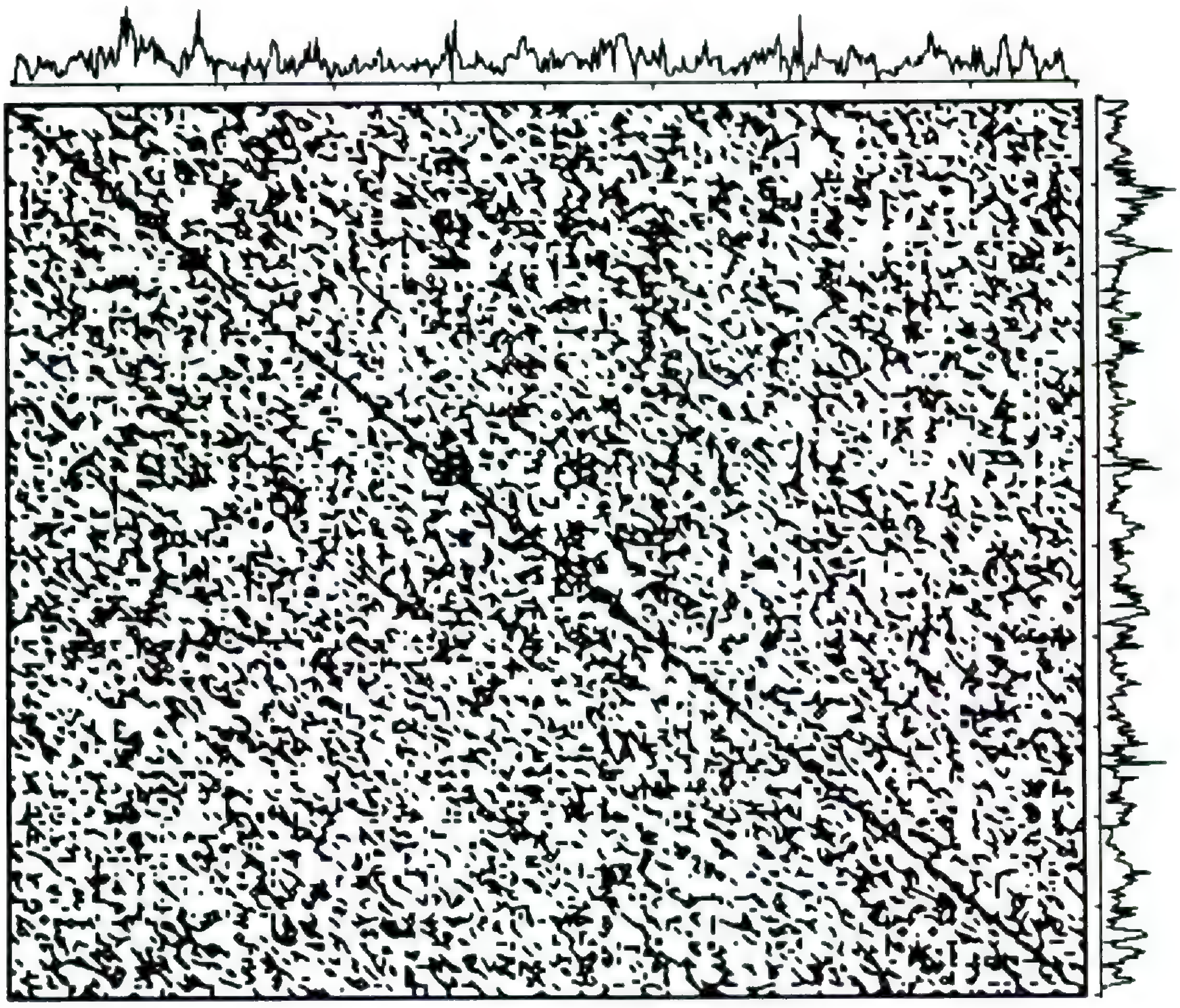


D. *Fragaria X ananassa*

FIGURE 2. Continued.

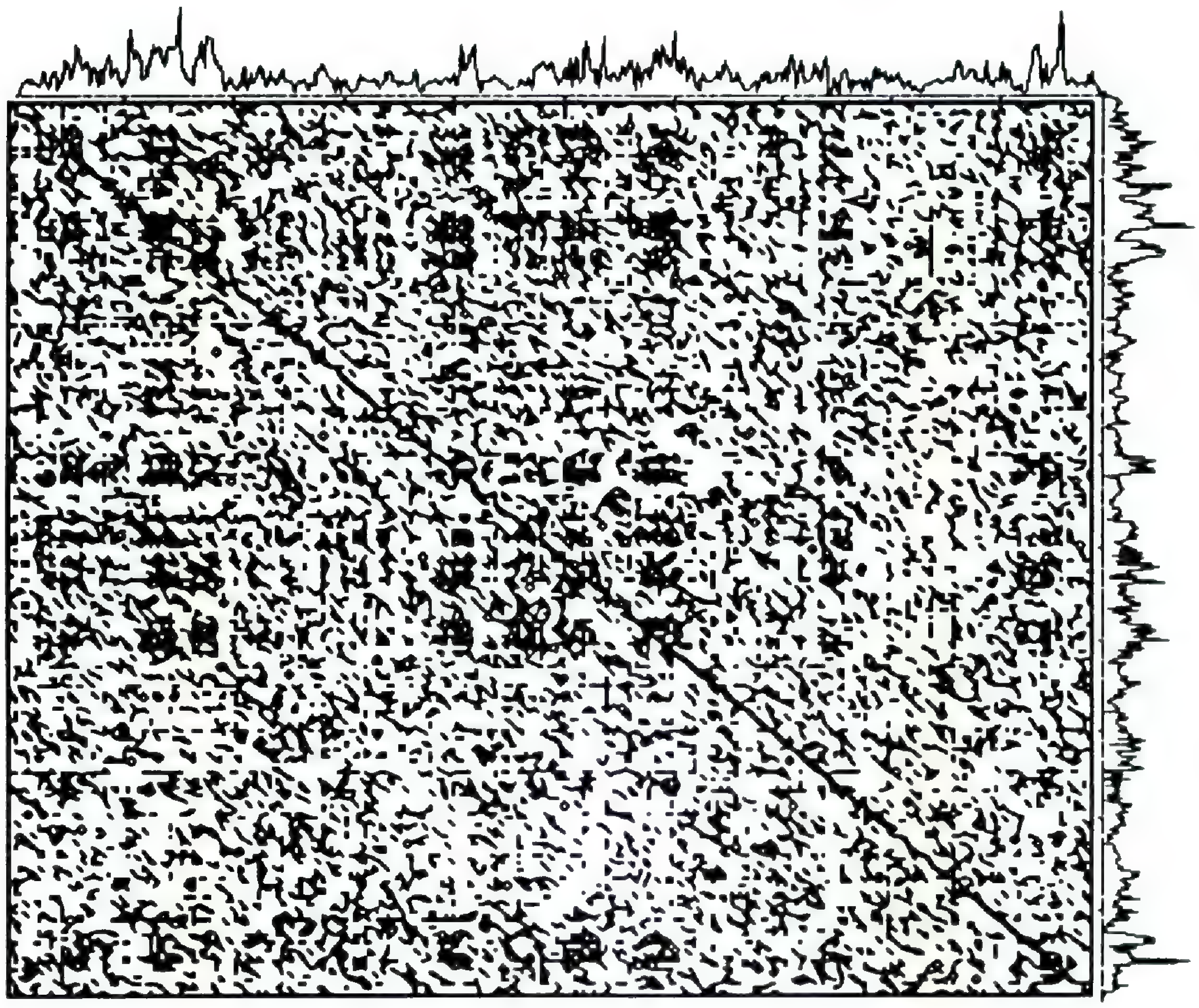


E. *Arabidopsis thaliana*

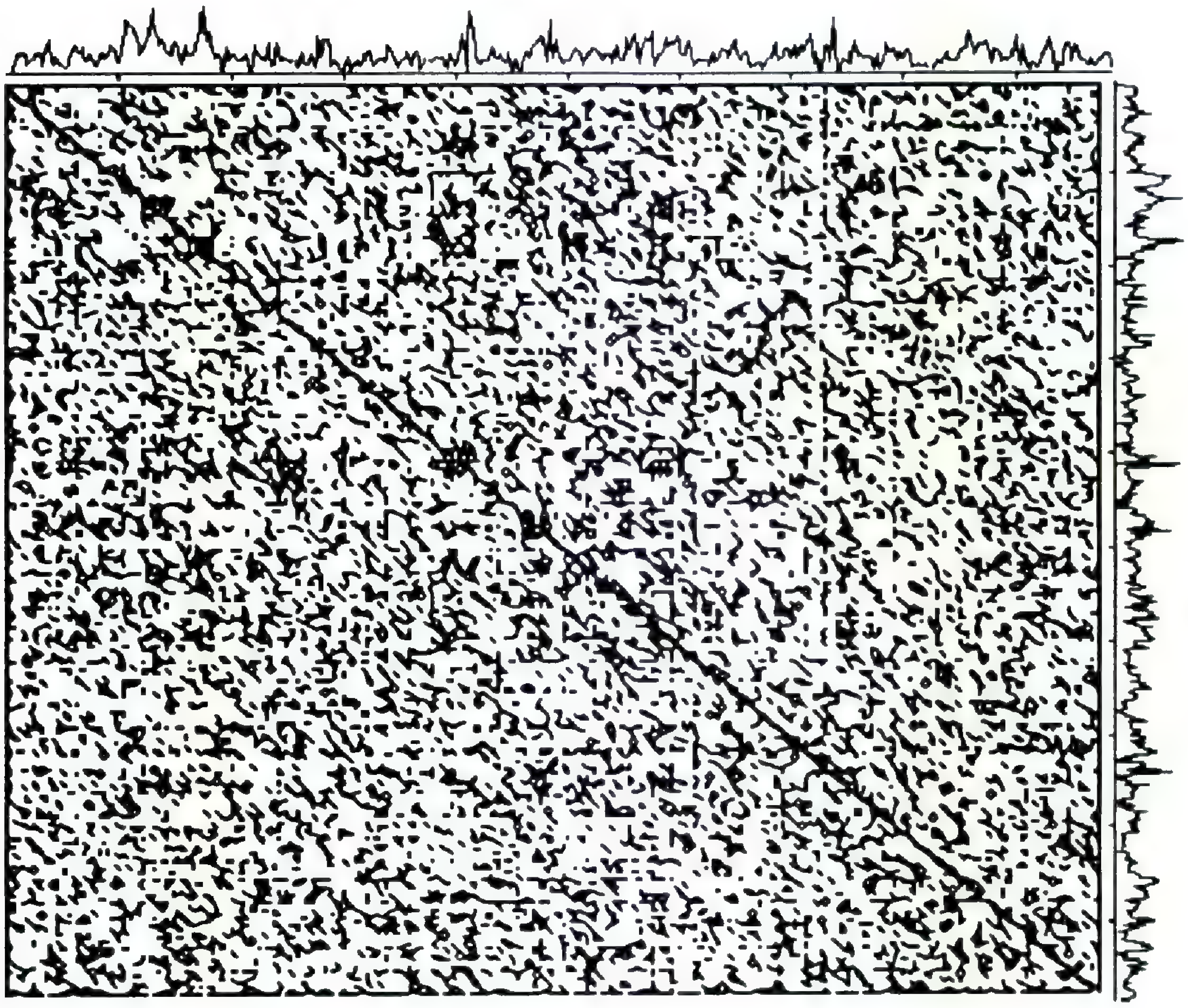


F. *Brassica napus*

FIGURE 2. Continued.

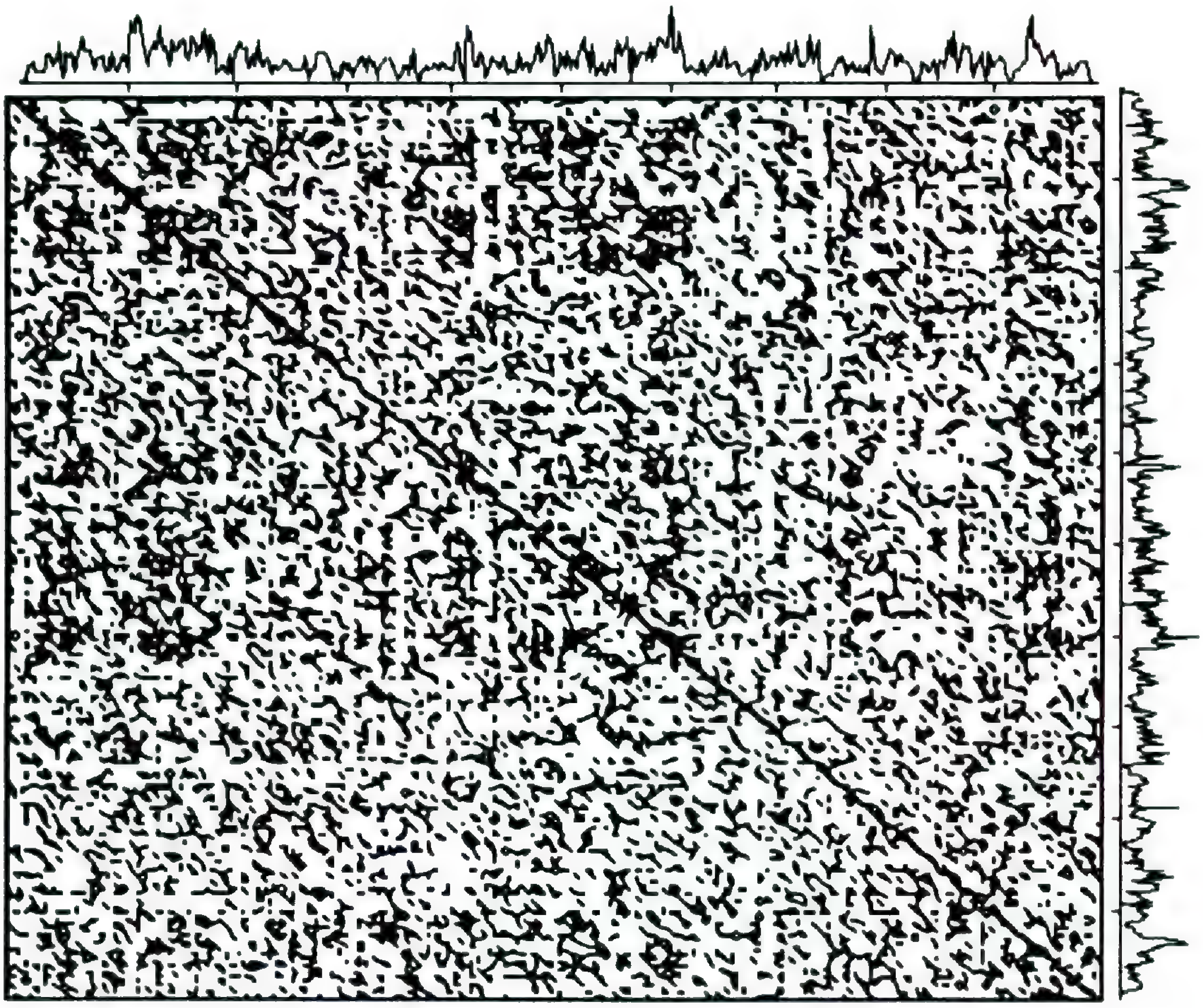


G. *Citrus limon*



H. *Sinapsis alba*

FIGURE 2. Continued.



I. *Lycopersicon esculentum*

FIGURE 2. Continued.

otide composition bias. Slippage-like mechanisms that lead to shuffling of small direct repeats in the expansion segments during turnover will obliterate evidence of ancestry in some cases, making homology assessment simply guesswork. This problem will be especially acute for deep divergences. Molecular coevolution among expansion segments will result in non-independence of characters. In their analysis of the secondary structure of the LSU in *Drosophila*, Hancock et al. (1988) described evidence for compensatory change among positions in the expansion segments. Weighting schemes have been proposed for compensatory mutations in the base-paired stem regions of rDNA based on secondary structure predictions (Wheeler & Honeycutt, 1988; Dixon & Hillis, 1993). Similar weighting schemes may be useful for expansion segments as well. The extent to which there is compensatory mutation in the expansion segments in plants is not known and awaits detailed modeling of secondary structure. In his molecular systematic work with the LSU of rDNA in salamanders, Larson (1991) explored the rates of sequence change in the expansion segments and concluded that violations to assumptions of parsimony were not severe enough to affect adversely the outcome of the analysis. He also proposed that a rate-invariant parsimony based on compositional statistics (Sidow & Wilson, 1990) be employed for phylogenetic analyses based on the LSU to compensate for the nucleotide composition bias observed in this molecule, especially for deep divergences. These post-alignment approaches, however, do not address the difficulties presented by the LSU for the basic assumptions of homology and independence among characters.

Because the number of plant species for which complete LSU sequence data currently are available is small and not representative of the overall phylogenetic diversity of the plant kingdom, we are sequencing the complete LSU of rDNA from representatives of all the major plant lineages. These data will enable us to determine if the patterns of sequence change described in this paper are found throughout the plant kingdom or are lineage-specific, and to test empirically the phylogenetic utility of both the expansion segments and the conserved core regions in the LSU of rDNA in plants.

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THE ITS REGION OF NUCLEAR RIBOSOMAL DNA: A VALUABLE SOURCE OF EVIDENCE ON ANGIOSPERM PHYLOGENY[1]

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Abstract

The internal transcribed spacer (ITS) region of 18S–26S nuclear ribosomal DNA (nrDNA) has proven to be a useful source of characters for phylogenetic studies in many angiosperm families. The two spacers of this region, ITS-1 and ITS-2 (each <300 bp), can be readily amplified by PCR and sequenced using universal primers, even from DNAs of herbarium specimens. Despite high copy numbers of both spacers, the near uniformity of ITS paralogues, attributed to rapid concerted evolution, allows direct sequencing of pooled PCR products in many species. Divergent paralogues, where detected, require cloning, but may offer a means of obtaining multiple estimates of organismal relationships and of determining placement of the root in a phylogeny independent of outgroup considerations. In reported studies, variation between ITS sequences is mostly attributable to point mutations. A relatively minor proportion of sites is affected by insertions or deletions (indels) among sequences that are similar enough to have retained sufficient signal for phylogenetic analysis. Within these limits, sequence alignment is generally unambiguous except in small regions of apparently lower structural constraint. Phylogenetic analyses of combined data sets from both spacers, where examined, yield trees with greater resolution and internal support than analyses based on either spacer alone. This beneficial effect of simultaneous analysis is not surprising based on the low number of useful characters in each spacer. This effect also suggests high complementarity of spacer data, in accord with similarity in size, sequence variability, and G + C content of ITS-1 and ITS-2 in most investigated groups of closely related angiosperms. Non-independent evolution of ITS sites involved in intraspacer RNA base-pairing may occur, given possible functional constraints, but preliminary secondary structure analyses of ITS-2 in *Calycadenia* (Asteraceae) show no definite evidence of compensatory spacer mutations. As expected, levels of ITS sequence variation suitable for phylogenetic analysis are found at various taxonomic levels within families, depending on the lineage. The apparent rates of ITS molecular evolution are roughly correlated with plant life-form, as with chloroplast DNA (cpDNA) data, but reasons for this observation are unclear. ITS characters have improved our understanding of angiosperm phylogeny in several groups by (1) corroborating earlier unexpected findings, (2) resolving conflicts between other data sets, (3) improving resolution of species relationships, or (4) providing direct evidence of reticulate evolution. Hybridization or sorting of ancestral polymorphism in a lineage can complicate interpretation of trees based on any type of evolutionary evidence, including ITS or cpDNA sequences, particularly in the absence of at least one independent phylogenetic data set from the same organisms. The need for phylogenetic markers from the nuclear genome, to complement the rapidly growing body of cpDNA data, makes the ITS region a particularly valuable resource for plant systematists.

Until recently, most plant systematists reserved DNA sequencing for phylogenetic analyses of taxa with sequences too divergent to be easily interpreted by restriction site mapping. Consequently, only moderately to slowly evolving DNA sequences have been used widely in plant phylogenetics (e.g., *rbc*L, see Chase et al., 1993; 18S and 26S nuclear ribosomal DNA, see Bult et al., 1995; Nickrent & Soltis, 1995). With the recent advent of polymerase chain reaction (PCR) technology, however, DNA

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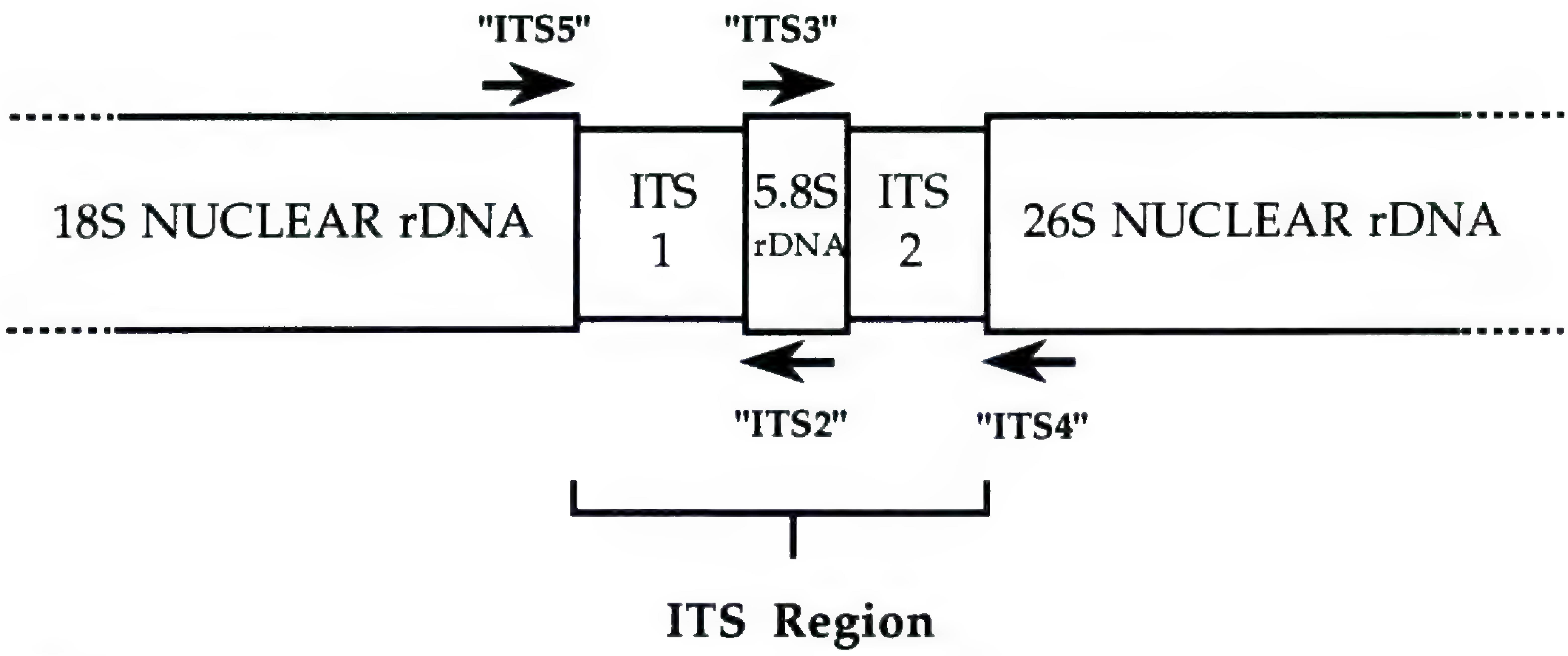


FIGURE 1. Organization of the ITS region. Arrows indicate orientation and approximate position of primer sites. Primer names (in quotation marks) and sequences are from White et al. (1990). Reprinted with permission from Baldwin (1993a).

sequencing is now sufficiently inexpensive and easy to use for phylogenetic studies at all taxonomic levels. This sequencing option offers increased precision by permitting better homology assessment of molecular characters and character-states than is possible by restriction site mapping.

The primary challenge to using nucleotide characters for lower-level phylogenetic studies is the identification of easily amplifiable and relatively rapidly evolving, but unambiguously alignable, DNA regions that can provide sufficient, suitable variation within a short sequence segment. There is an especially great need for such data from the nuclear genome, to supplement the wealth of chloroplast DNA (cpDNA) restriction site information that has accumulated over the past decade. This need is made particularly evident by an increasing number of reports of (1) strong conflict between cpDNA trees and relationships based on other lines of evidence, often explained by interspecific hybridization (reviewed in Rieseberg & Soltis, 1991; Rieseberg & Brunsfeld, 1992; Rieseberg & Wendel, 1993) or lineage sorting (see Pamilo & Nei, 1988; Doyle, 1992), and (2) insufficient phylogenetic resolution in cpDNA trees, with information often concentrated along a limited subset of internal tree branches. These findings do not detract from the value of cpDNA phylogenetic data but merely demonstrate the potential inadequacies of any estimate of organismal phylogeny based on a single organellar genome or nuclear gene (see Doyle, 1992).

In this paper we evaluate the phylogenetic utility of a genomic region that has attracted increased attention among those interested in applying nuclear-DNA sequencing analysis to lower-level phylogenetic questions in angiosperms (and other eukaryotes): the internal transcribed spacer (ITS) region of 18S–26S nuclear ribosomal DNA (nrDNA). This region includes three components as defined here (Fig. 1): the 5.8S subunit, an evolutionarily highly conserved sequence, and, most importantly from the perspective of this paper, two spacers designated ITS-1 and ITS-2. As testimony to the recent high level of interest in the ITS region, ongoing plant phylogenetic studies using ITS-1 and ITS-2 vastly outnumber those few that are published in detail. We therefore have relied largely on our own published and unpublished work with these sequences for most of the examples and comparisons presented here.

FUNCTIONAL CONSIDERATIONS

The ITS region is part of the transcriptional unit of nrDNA, but the spacer segments of the transcript are not incorporated into mature ribosomes. Instead, ITS-1 and ITS-2 regions of the nrDNA transcript appear to function, at least in part, in the maturation of nrRNAs. In vivo mutational analyses in yeast (*Saccharomyces cerevisiae*) indicate that deletions of certain regions within ITS-1 can inhibit production of mature small and large subunit rRNAs (Musters et al., 1990; van Nues et al., 1994), whereas certain deletions or point mutations in ITS-2 prevent or reduce processing of large-subunit rRNAs (van der Sande et al., 1992). Intrastrand Watson-Crick base pairing within spacers and perhaps between spacer and precursor nrRNA sequences may bring the subunit boundaries into close proximity within a processing domain (Gonzalez et al., 1990a; Thweatt & Lee, 1990; Venkateswarlu & Nazar, 1991; Melekhovets et al.,

1994). Given this hypothesized role, much like that of self-splicing group I introns, it seems probable that ITS-1 and ITS-2 are under some evolutionary constraint in structure and sequence, as suggested by size and G + C (guanine + cytosine) content comparisons among angiosperms (see Table 1 and below).

Evolutionary Considerations for Phylogeny Reconstruction

PRELIMINARY INSIGHTS

Early attempts at alignment of ITS sequences across angiosperm families indicated that plant ITS-1 and ITS-2 sequences have diverged more at the nucleotide level than have the nrDNA subunits (Yokota et al., 1989), with the exception of subregions in expansion segments or divergent domains of the large (26S) subunit (Hassouna et al., 1984; Cullings & Bruns, 1992; C. Bult, pers. comm.). In addition, nrDNA restriction site studies of closely related plant species have shown consistently that a high proportion of variable sites map to the ITS region, as well as to the intergenic spacer (IGS) and external transcribed spacer (ETS) (e.g., Appels & Dvořák, 1982; Sytsma & Schaal, 1985; Kim & Mabry, 1991). Such studies, however, have only rarely detected within-genome length variation in the ITS region (but see Sytsma & Schaal, 1985, 1990; and Jorgensen & Cluster, 1988), in contrast to repeated documentation of such length variants in the IGS (e.g., Rogers & Bendich, 1987; Schaal & Learn, 1988). These preliminary indications of ITS length conservation and high ITS nucleotide sequence variability suggested that DNA sequences of these spacers might be readily alignable across closely related taxa, yet sufficiently variable to allow resolution of lower-level phylogenetic questions in angiosperms. Such properties were evident in ITS sequences from Oomycetes (Lee & Taylor, 1992) and hominoids (Gonzalez et al., 1990b) prior to attempts by angiosperm systematists to utilize these spacers. These considerations were a major stimulus to initial exploration of the ITS region for use in plant phylogenetic studies (Baldwin, 1992).

FAVORABLE PROPERTIES

Several general features of the ITS region promote its use for phylogenetic analyses of angiosperms. First, along with the other components of the nrDNA multigene family, the ITS region is highly repeated in the plant nuclear genome. The entire nrDNA repeat unit is present in up to many thousands of copies arranged in tandem repeats at a chromosomal locus or at multiple loci (Rogers & Bendich, 1987; reviewed in Hamby & Zimmer, 1992). This high copy number promotes detection, amplification, cloning, and sequencing of nrDNA.

Second, and most importantly from the standpoint of phylogeny reconstruction, this gene family undergoes rapid concerted evolution (e.g., Arnheim et al., 1980; Appels & Dvořák, 1982; Arnheim, 1983; Hillis et al., 1991; and see Zimmer et al., 1980), via unequal crossing-over and gene conversion. This property promotes intragenomic uniformity of repeat-units, in some cases even between nrDNA loci on non-homologous chromosomes (e.g., Arnheim et al., 1980; Arnheim, 1983; Hillis et al., 1991; Wendel et al., 1995), and, in general, promotes accurate reconstruction of species relationships from these sequences (Hamby & Zimmer, 1992; Sanderson & Doyle, 1992). As a result, direct sequencing of pooled nrDNA PCR products can be used to extract phylogenetic information in many species. In addition, concerted evolution and sexual recombination may promote nrDNA uniformity within interbreeding populations and thereby minimize the importance of intrapopulational sampling in phylogenetic studies, e.g., as in *Lomatium grayi* Coult. & Rose and *L. laevigatum* Coult. & Rose (Soltis & Kuzoff, 1993), and *Limonium* (M. Hamilton, pers. comm.).

Third, the small size of the ITS region (<700 bp in angiosperms) and the presence of highly conserved sequences flanking each of the two spacers make this region easy to amplify, even from herbarium material, using universal eukaryotic primers designed by White et al. (1990) (Fig. 1). In many cases, these primers can be used to generate single-stranded DNA for sequencing directly from genomic DNA, bypassing a separate double-stranded DNA amplification procedure (Baldwin, 1992, 1993a; Wojciechowski et al., 1993). We have used this approach, and even more streamlined double-stranded sequencing methods (e.g., cycle-sequencing), to generate high-quality ITS sequences in several angiosperm groups (see below), generally without evidence of divergent repeat-types within individuals.

DIVERGENT PARALOGUES

If concerted evolution fails to homogenize ITS paralogues (e.g., those at different chromosomal loci) through a succession of speciation events, the possibility of unknowingly sampling sequences with different evolutionary histories is a real danger to phylogenetic analysis (Fitch, 1970; Sanderson & Doyle, 1992). On the other hand, if such paralogues are retained in most or all members of a

TABLE 1. Sizes and percent G + C content of ITS-1 and ITS-2 (see Fig. 1) of angiosperm taxa. ITS-1 and ITS-2 boundaries chosen by various authors may differ by up to a few base pairs. NA = data not available.

	ITS-1		ITS-2	
Taxon	Size (bp)	% G + C	Size (bp)	% G + C
Adoxaceae, *Viburnum*, 28 spp. (Donoghue & Baldwin, 1993)	224–231	59–69	215–227	59–69
Apiaceae, *Daucus*, 1 sp. (Yokota et al., 1989)	215	49	224	52
Apiaceae, Apioideae, 42 spp. (S. Downie, pers. comm.)	204–221	49–58	215–226	43–61
Asteraceae, Madiinae, 8 gen., 45 spp. (Baldwin, 1992, 1993a; Baldwin & Robichaux, 1995)	254–261	48–51	216–223	50–53
Asteraceae, Lactuceae, 6 gen., 11 spp. (Kim & Jansen, 1994)	246–253	52–54	220–222	53–58
Betulaceae, *Alnus*, 3 spp. (Savard et al., 1993)	215–216	58–64	228–229	53–65
Betulaceae, *Betula*, 3 spp. (Savard et al., 1993)	214–219	62–63	226–231	63–65
Betulaceae, *Ostrya*, 1 sp. (Savard et al., 1993)	215	61	227	62
Brassicaceae, *Arabidopsis*, 1 sp. (Unfried & Gruendler, 1990)	268	57	187	55
Brassicaceae, *Sinapis*, 1 sp. (Rathgeber & Capesius, 1989)	265	51	188	54
Canellaceae, *Canella*, 1 sp. (Suh et al., 1992)	272	63	209	63
Cucurbitaceae, *Cucumis*, 1 sp. (Kavanagh & Timmis, 1988)	216	56	237	60
Cucurbitaceae, *Cucurbita*, 2 sp. (Torres et al., 1990)	187–229	51–60	245–252	54–66
Fabaceae, Galegeae, 4 gen., 31 spp. (Wojciechowski et al., 1993)	221–231	55–60	207–217	50–54
Fabaceae, *Vicia*, 1 sp. (Yokota et al., 1989)	235	52	208	50
Fabaceae, *Vigna*, 1 sp. (Schiebel & Hemleben, 1989)	205	60	220	59
Onagraceae, *Epilobium*, 22 spp. (Baum et al., 1994)	240–244	53–62	211–216	54–60
Malvaceae, *Gossypium*, 1 sp. (Mukhamedov, 1992)	287	58	229	60
Malvaceae, 7 gen. (Ray, 1994a, pers. comm.)	277–298	49–53	240	57
Poaceae, Pooideae, 6 gen., 10 spp. (Hsiao et al., 1994)	217–223	55–64	213–221	59–67
Poaceae, *Sorghum*, 1 sp. (Hsiao et al., 1994)	207	56	217	67
Poaceae, *Oryza*, 1 sp. (Takaiwa et al., 1985)	194	73	233	77
Polemoniaceae, 18 gen., 38 spp. (Porter, 1993b)	242–262	42–65	187–195	48–62
Ranunculaceae, 7 gen., 27 spp. (Hodges & Arnold, 1994)	214–246	NA	198–216	NA
Rosaceae, *Fragaria*, 1 sp. (Simovic et al., 1990)	~249	68	207	65
Rosaceae, Maloideae, 20 gen., 20 spp. (Campbell et al., 1993a)	208–221	65–72	211–224	67–72

TABLE 1. Continued.

Taxon	ITS-1		ITS-2	
	Size (bp)	% G + C	Size (bp)	% G + C
Rosaceae, *Prunus*, 1 sp. (Campbell et al., 1993a)	242	62	209	69
Rosaceae, *Rosa*, 1 sp. (Campbell et al., 1993a)	249	60	207	57
Rosaceae, *Spiraea*, 1 sp. (Campbell et al., 1993a)	251	65	230	70
Salicaceae, *Populus*, 1 sp. (D'Ovidio, 1992)	214	67	207	70
Saxifragaceae, 9 gen., 28 spp. (Soltis & Kuzoff, in press)	256–267	NA	224–238	NA
Scrophulariaceae, *Mimulus*, 8 spp. (Ritland et al., 1993)	189–214	44–49	203–225	45–48
Solanaceae, *Lycopersicon*, 1 sp. (Kiss et al., 1988)	217	68	217	71
Solanaceae, *Nicotiana*, 1 sp. (Venkateswarlu & Nazar, 1991)	216	69	217	65
Styracaceae, *Styrax*, 19 spp. (Fritsch, 1994a)	255–264	NA	208–221	NA
Viscaceae, *Arceuthobium*, 22 spp. (Nickrent et al., 1994)	208 (mean)	ca. 31–36	226 (mean)	ca. 30–37
Winteraceae, 7 gen., 11 spp. (Suh et al., 1993)	235–252	NA	213–226	NA

species lineage, thorough sampling of these sequences can offer independent estimates of organismal phylogeny (Suh et al., 1993; see Sanderson & Doyle, 1992) and a means of rooting that portion of the tree in the absence of outgroup data (Iwabe et al., 1989). In other words, non-homogenized paralogues can present opportunities along with some potential danger. Cloning and sequencing of individual repeat-types may be necessary, however, to recover any useful information from such nrDNAs (see Ritland et al., 1993).

A direct sequencing approach can sometimes aid in detection of intragenomic repeat-type variants, which can be tentatively diagnosed by two or more nucleotide states at a site, implying superimposition of two or more sequence patterns. Persistence of the same apparent site-polymorphism(s) in ITS sequences of both complementary DNA strands reduces the likelihood that the multiple bands are attributable to a sequencing artifact, e.g., self-pairing of single-stranded sequencing products.

ITS variants within individuals that differ by an insertion or deletion (indel) of one or more nucleotides can be more readily detected from direct sequences than variants that differ by point mutations. At and above (i.e., in the direction away from the sequencing primer) an indel on a sequencing gel, a high proportion of sites will show two different states (bands). Sequence of the complementary strand will show the reverse pattern, i.e., will be unambiguous through the region that showed extensive polymorphism in the opposing sequence up to the other boundary of the indel, above which extensive site-polymorphism will be observed. Aside from identification of length-variant repeat-types, direct sequencing of both complementary strands can provide unambiguous sequence of the shortest repeat-type without cloning, assuming that the ITS variants differ by only one indel between the sequencing-primer sites.

It is still arguable whether sequencing of individual PCR clones or of pooled PCR products is the better approach to phylogenetic analysis of the ITS region, or of nrDNA in general. Routine sequencing of large numbers of individual PCR clones from each genomic DNA is ideal, but may be prohibitively expensive and labor-intensive for large-scale phylogenetic studies. Sequencing of only one PCR clone per reaction risks sampling of misreplicated products and, of course, undersampling of any repeat-type variants. In contrast, the small error rate of *Taq* polymerase dictates that the predominant signal in direct sequences of pooled PCR products is likely to represent DNAs that were accurately replicated (Saiki et al., 1988; Gyllensten & Erlich, 1988; Bruns et al., 1990).

The relative merits of sequencing cloned versus pooled PCR products for initial detection of variable repeat-types are less clear. Sequencing of several PCR clones may be necessary to detect repeat-type variants of similar size that are readily apparent from direct sequences of pooled PCR products, e.g., in the polyploid *Raillardella scaposa* (A. Gray) A. Gray (Asteraceae) (Baldwin, Wojciechowski & Donoghue, unpublished). Conversely, sequencing of only two PCR clones per reaction revealed widely-divergent ITS repeat-types in four of six species of Winteraceae that potentially possessed these two sequence classes of similar-sized ITS variants (Suh et al., 1993). Direct ITS-1 and ITS-2 sequences from pooled PCR products of two of these four species (*Bubbia comptonii* (E. G. Baker) Dandy and *Zygogynum bicolor* V. Tieghem, genomic DNAs kindly provided by Y. Suh) each showed evidence of only one repeat-type (Baldwin & Donoghue, unpublished), which corresponded to the orthologous "*Bubbia* 2" and "*Zygogynum bicolor* 2" sequences (Suh et al., 1993). Use of different PCR primers and reaction conditions by Suh et al. (1993) and Baldwin & Donoghue (unpublished) may account for lack of evidence of the other pair of orthologues in the direct sequences, e.g., by sequence divergence between the two classes of repeat-types at a primer annealing-site used by Baldwin & Donoghue. If the relative concentration of repeat-types in pooled PCR products is even crudely reflected by sequencing-autoradiograph band intensities, then direct sequencing of pooled PCR products should indicate whether ITS variants are present in sufficient concentration to allow cloning without extensive screening of recombinants. Most angiosperm workers have routinely taken such an initial direct sequencing approach (but see Suh et al., 1993). Denaturing gradient gel electrophoresis (DGGE), which allows separation of PCR products that differ by as little as one nucleotide substitution, has been used successfully as an alternative method for detecting ITS variants (R. K. Jansen, pers. comm.) that could potentially be gel-isolated and sequenced directly.

At a more fundamental level, PCR "selection" and "drift" (Wagner et al., 1994) can potentially lead to a pool of PCR products of the ITS region that includes a biased representation of the distinctive paralogues present in a genome. To ameliorate the effects of these processes, Wagner et al. (1994) recommend reducing the amount of genomic DNA in PCR, reducing the number of PCR cycles, and pooling products of multiple PCRs. Artifacts from PCR "jumping," i.e., chimeric sequence products formed by incomplete PCR products acting as PCR primers on heterologous sequences (Saiki et al., 1988; Scharf, 1990), may also be reduced by application of these recommendations. The possibilities of PCR "drift" and PCR "jumping" provide increased impetus for sequencing both strands of the ITS region from products of different PCRs.

An additional, follow-up strategy for detection of paralogues that may affect phylogeny reconstruction is to screen genomic DNAs of the study species for presence or absence of any restriction sites that diagnose the major ITS clades indicated by phylogenetic analysis of direct or cloned sequences of PCR products. Such screening can be accomplished by Southern blot hybridization of electrophoresed genomic DNA digests using labeled, PCR-amplified nrDNA sequences from the study group or, in some cases, labeled clones of the entire nrDNA repeat unit. Additivity for restriction patterns that define different ITS clades in a single genomic DNA can provide a reevaluation of orthology/paralogy relationships of ITS sequences. This strategy was recently used by Wendel (1995) to determine whether both parental ITS repeat types occur in allopolyploid species of *Gossypium*.

Technical Considerations for ITS Sequencing

METHODS

In general, we have followed the asymmetric-PCR and sequencing procedures detailed in Baldwin (1992), which typically yield excellent results with high-purity DNAs (see below). We have also successfully used symmetric-PCR (with primers in equimolar, 10mM, concentrations, otherwise as in Baldwin, 1992) and cycle-sequencing methods, using ^{33}P (dsDNA cycle sequencing system, BRL-Gibco, Gaitherburg, Maryland), ^{35}S (Cyclist, Stratagene, La Jolla, California), or fluorescent dyes (with automated sequencing) for labeling. For amplification of DNAs from old herbarium specimens (up to at least 80 years old) we have achieved much greater success with the PCR reaction buffer described in Pääbo (1990) than with standard buffers. The ease of ITS amplification from herbarium specimens makes sampling of extinct, rare, or inaccessible populations practical and can increase the feasibility of comprehensive species sampling within widespread plant groups (see Loockerman & Jansen, in press, for a review of DNA extraction protocols for herbarium material).

DNA PURITY

High purity of genomic DNAs or PCR products can be critical for successful ITS sequencing. In our experience, most ITS sequencing problems in plants that are not resolved by changes in sequencing-reaction or gel-electrophoresis conditions can be solved by further purification of genomic DNAs (e.g., on cesium chloride gradients) or PCR products (e.g., by the Geneclean procedure, Bio 101, La Jolla, California). Template purity appears to be more important for ITS sequencing than for *rbc*L sequencing, based on communications with other workers.

SECONDARY STRUCTURE FORMATION

Sequencing of the ITS region can be complicated by within-strand Watson-Crick base pairing that may interfere with *Taq* polymerase activity or may alter the electrophoretic mobility of DNA fragments in sequencing gels. Spacer segments with G + C richness or any substantial intrastrand complementarity may form secondary structures under some reaction conditions. Such intramolecular pairing is expected based on the probable functional behavior of transcripts of these sequences (see "Functional Considerations"). For this reason, high-temperature sequencing reactions with *Taq* polymerase (or another thermophilic DNA polymerase) and use of dGTP analogs, such as dITP or deaza-dGTP, in sequencing reactions (and even in PCR amplifications) are recommended. Use of DMSO (5–10%), formamide, or tergitol in PCRs and sequencing reactions can also be useful for overcoming such problems (Winship, 1989; Zhang et al., 1992; Bechmann et al., 1990; and see Kim & Jansen, 1994).

ITS Sequence Comparisons in Angiosperms

In this paper, we focus primarily, but not exclusively, on results from our own ITS phylogenetic studies within five dicotyledon families: Adoxaceae (*Viburnum*: Donoghue & Baldwin, 1993, unpublished), Asteraceae (Madiinae: Baldwin, 1991, 1992, 1993a, b, 1994, unpublished; Baldwin & Robichaux, 1995), Fabaceae (Galegeae: Wojciechowski et al., 1993; Sanderson & Wojciechowski, 1993, unpublished; Sanderson & Liston, in press), Polemoniaceae (Porter, 1993a, b, 1994, unpublished), and Rosaceae (Maloideae: Campbell et al., 1993a, b, in press). A number of other ITS studies have been published or are in press, including Baum et al. (1994: *Epilobium*, Onagraceae), Fritsch (1994a, see 1994b: *Styrax*, Styracaceae), Hodges & Arnold (1994: *Aquilegia*, Ranunculaceae), Hsiao et al. (1994: Pooideae, Poaceae), Kim & Jansen (1994: *Krigia*, Asteraceae), Miller (1994: *Silene*, Caryophyllaceae), Nickrent et al. (1994: *Arceuthobium*, Viscaceae), Ray (1994a, see Ray, 1994b: *Lavatera* and *Malva*, Malvaceae), Ritland et al. (1993: *Mimulus*, Scrophulariaceae), Savard et al. (1993: *Alnus* and *Betula*, Betulaceae), Sang et al. (1994b, 1995: *Dendroseris* and *Robinsonia*, Asteraceae), Soltis & Kuzoff (in press: *Heuchera* group, Saxifragaceae), Suh et al. (1993: Winteraceae), and Sun et al. (1994: *Sorghum*, Poaceae).

Many other ITS phylogenetic investigations of angiosperms are, however, in progress. Some of these studies are partially documented by published abstracts, including work on Apiaceae (Downie et al., 1994; Hardig & Soltis, 1994), Apocynaceae (Sper-Whitis et al., 1992), Asteraceae (Urbatsch & Baldwin, 1993; Bain & Jansen, 1994; Kim et al., 1994), Capparaceae (Hahn et al., 1994), Cornaceae (Murrell, 1994), Ericaceae (Hileman et al., 1994; Kron & King, 1994; Markos & Parker, 1994), Fouquieriaceae (Schultheis & Baldwin, 1994), Lemnaceae (Les et al., 1994), Liliaceae sensu lato (Bogler & Simpson, 1993, 1994; Bogler, 1994a, b; Bogler et al., 1994), Nothofagaceae (Manos, 1993), Onagraceae (Hahn & Sytsma, 1993), Poaceae (Kellogg et al., 1994; Mason et al., 1994), Paeoniaceae (Sang et al., 1994a), Polemoniaceae (Johnson et al., 1994), and Portulacaceae and related families (Hershkovitz & Zimmer, 1994). As these new studies are published our conclusions based on a relatively limited sample of angiosperms will no doubt require some adjustment. Nevertheless, sufficiently clear patterns have emerged to warrant some tentative generalizations.

LENGTH VARIATION

Length conservation of ITS-1 and ITS-2, as seen in earlier plant studies (see Baldwin, 1992), remains apparent from the expanded perspective of more recent investigations (Table 1). Sizes of ITS-1 and ITS-2 in these newly obtained sequences fall nearly within the narrow range reported previously for other angiosperms. In all flowering plants reported to date ITS-1 and ITS-2 are each less than 300 bp (ITS-1: 187 to 298 bp, ITS-2: 187 to 252 bp), in contrast to much longer spacers in some other eukaryotes, e.g., some vertebrates (Stewart et al., 1983; Goldman et al., 1983; Gonzalez et al., 1990b). Given that the 5.8S subunit is almost invariant in length in reported angiosperms (mostly 163 or 164 bp), the entire ITS region (Fig. 1) appears to be universally under 700 bp in flowering plants.

Relative sizes of the two spacers vary between

TABLE 2. Pairwise sequence divergence, potential phylogenetic information, and prevalence of gap-containing nucleotide positions in ITS-1 and ITS-2 sequences of angiosperms.

Taxa	Percent pairwise sequence divergence				Potential informative sites/aligned sites (%)		% of aligned sites with >1 gap ITS-1
	ITS-1		ITS-2				
(number of species)	Range	Mean (SD)	Range	Mean (SD)	ITS-1	ITS-2	+ 2
Hawaiian Madiinae, 3 gen. (25) (Baldwin & Robichaux, in press)	0–6.2	2.8 (± 1.2)	0–5.9	2.9 (± 1.5)	23/257 (9)	22/221 (10)	<1
Calycadenia/Osmadenia (12) (Baldwin, 1993a)	0–12.5	5.8 (± 3.5)	0–10.0	4.6 (± 2.6)	37/257 (14)	27/223 (12)	2
California/Hawaiian Madiinae, 7 gen. (11) (Baldwin, 1992)	0.4–21.0	12.1 (± 4.4)	0.5–14.1	8.7 (± 3.0)	46/259 (18)	23/206 (11)	5
Fouquieria (11) (Schultheis & Baldwin 1994)	0–7.8	4.0 (± 1.8)	0.4–7.1	3.5 (± 1.7)	14/259 (5)	15/227 (7)	3
Astragalus (26) (Wojciechowski et al., 1993)	0–10.2	3.7 (± 3.1)	0–8.8	3.7 (± 1.5)	22/238 (9)	22/215 (10)	7
Viburnum (28) (Donoghue & Baldwin, 1993)	0–13.6	7.3 (± 2.3)	0–11.9	6.1 (± 2.1)	43/239 (18)	36/229 (16)	5
Galegeae, 17 gen. (28) (Sanderson & Wojciechowski, 1993)	0–37.8	13.3 (± 8.3)	0–33.7	10.6 (± 8.5)	95/269 (35)	76/237 (32)	15
Gilia sect. *Gilliandra* (16) (Porter, 1993a, b)	0–13.9	5.7 (± 2.8)	0–12.5	5.4 (± 2.5)	30/256 (12)	28/188 (15)	6
Polemoniaceae, 19 gen., (28) (Porter, 1993a, b)	0–39.0	18.0 (± 7.3)	0–29.5	14.6 (± 5.5)	162/301 (54)	113/228 (50)	40
Maloideae, 20 gen. (20) (Campbell et al., 1993a, in press)	0–16.6	8.9 (± 3.1)	3.3–24.1	14.8 (± 3.7)	45/234 (19)	72/235 (31)	16

and, in at least some cases, within families, with little indication of a broad-scale phylogenetic pattern. ITS-1 is consistently longer that ITS-2 (or rarely equal in length) in all available sequences of Adoxaceae, Asteraceae, Brassicaceae, Canellaceae, Malvaceae, Onagraceae, Polemoniaceae, Ranunculaceae, Salicaceae, Saxifragaceae, Styracaceae, and Winteraceae. Both reported Brassicaceae sequences show a larger size disparity between ITS-1 and ITS-2 (77 bp in *Sinapis alba* L.; 81 bp in *Arabidopsis thaliana* (L.) Heynh.; Table 1) than in any other angiosperm sequences. Conversely, ITS-2 is larger than ITS-1 in all sequences of Betulaceae, Cucurbitaceae, Scrophulariaceae, and Viscaceae reported to date. The two spacers are nearly equal in length in both available sequences from Solanaceae. In the large families Fabaceae, Poaceae, and Rosaceae, ITS-1 may be longer or shorter than ITS-2.

SEQUENCE DIVERGENCE COMPARISONS

In most investigated groups of angiosperms, ITS-1 pairwise divergence values are similar on average to those of ITS-2 (e.g., Table 2). The greatest disparity in divergence comparisons of ITS-1 and ITS-2 is in Asteraceae. Distances between ITS-1 sequences of taxa in the Hawaiian silversword alliance (Madiinae) are generally similar to ITS-2 distances from the same species comparisons (Baldwin, unpublished). In *Krigia* (Lactuceae), however, ITS-1 pairwise divergence values are, on average, twice those of ITS-2 (Kim & Jansen, 1994). Other reported Asteraceae fall

largely between these extremes. Average pairwise distances between ITS-1 sequences exceed those between ITS-2 sequences by 1.3 to 1 in *Calycadenia* (Baldwin, 1993a) and 1.5 to 1 in a broader survey of Madiinae (Baldwin, 1992). Outside Asteraceae, *Epilobium* (Onagraceae, Baum et al., 1994) and *Gossypium* (Malvaceae, J. Wendel, pers. comm.), like *Krigia*, show much higher pairwise divergence between ITS-1 sequences than between sequences of ITS-2. Such comparisons in most other flowering plant families suggest more equitable distribution of variation between the spacers. In these families, interspecific distances are only slightly higher on average between ITS-1 sequences than between sequences of ITS-2 (1 : 1 to 1.4 : 1), or, in *Arceuthobium* (Viscaceae: Nickrent et al., 1994), the *Heuchera* group (Saxifragaceae; Soltis & Kuzoff, in press), Maloideae (Rosaceae; Campbell et al., 1993a, in press), and *Nothofagus* (Nothofagaceae; Manos, 1993, pers. comm.), somewhat greater divergence exists between ITS-2 sequences than between those of ITS-1.

It should be noted, however, that we do not consider comparison of average pairwise distance values to be an adequate measure of relative evolutionary rates. Relative rate tests (e.g., Muse & Weir, 1992) are better suited for this purpose. Average distances are based on a set of non-independent comparisons because they do not take phylogenetic relationships into account. This non-independence casts doubt on the statistical significance of differences in distance values. Furthermore, average distances mask variance in relative distances, which is considerable in these studies.

From a finer-scale perspective, likelihood ratio heterogeneity tests conducted by Gaut & Weir (1994) suggest that there have been differences in rates of nucleotide substitution between small (57 bp) subregions within ITS-1 and ITS-2 in species of Compositae sampled by Baldwin (1992). The existence of rate heterogeneity between small ITS sequence partitions in these species is further suggested by the somewhat irregular distribution of ITS substitutions calculated across parsimony trees of these taxa (Fig. 2). Patterns of conserved and variable ITS subregions consistently similar to those in Figure 2 are not obvious from examination of mutational distributions in other plant groups we have studied (data not shown).

POTENTIAL PHYLOGENETIC INFORMATION IN ITS-1 AND ITS-2

Comparison of the proportions of aligned sites that are potentially informative for phylogenetic analysis in ITS-1 and ITS-2 (e.g., in Table 2) suggests a distribution of variation between the spacers that is similar to that estimated from raw divergence values. Similar percentages of aligned nucleotide positions are potentially informative in ITS-1 and ITS-2 (0.8 : 1 to 1.3 : 1) in most reported studies of angiosperms. In two investigations of Asteraceae (Baldwin, 1992; Kim & Jansen, 1994), in *Epilobium* (Onagraceae; Baum et al., 1994), and in *Gossypium* (Malvaceae; Wendel, 1995), however, ITS-1 included 50 to 100% more potentially informative characters than ITS-2. In one of these studies (Baldwin, 1992), however, variation is somewhat underestimated in ITS-2 because 17 highly variable ITS-2 sites of ambiguous alignment were excluded from analysis, as opposed to only 9 such sites in ITS-1. In contrast to the results from Asteraceae, *Epilobium*, and *Gossypium*, ITS-1 contained only 61% as many potentially informative sites as ITS-2 in an analysis of Maloideae (Rosaceae; Campbell et al., 1993a, in press).

As expected, resolution in phylogenies based on data from ITS-1 or ITS-2 alone is generally well correlated with the extent of variation within each spacer suggested from divergence values and numbers of potentially informative sites. Strict consensus trees based on ITS-1 data generally showed more complete phylogenetic resolution than those based on ITS-2 sequences in groups wherein greater pairwise distances and higher numbers of potentially informative sites were found in ITS-1 than in ITS-2 (e.g., Baldwin, 1992, 1993a; Porter, 1993a, b). The reverse was generally true as well: strict consensus trees based on ITS-2 data showed better resolution than those based on ITS-1 data in groups wherein higher pairwise distances and higher numbers of potentially informative sites were found in ITS-2 than in ITS-1 (e.g., Manos, 1993, pers. comm.; Soltis & Kuzoff, in press). In all of these examples, however, trees based on either spacer alone provided at least some resolution of relationships that were unresolved or weakly supported in trees based on the other spacer.

In our studies, complementarity of ITS-1 and ITS-2 data sets was further indicated by more complete and robust resolution in trees based on combined spacer data than in trees based on either ITS-1 or ITS-2 alone. These results are not surprising given approximate doubling of the number of characters in the analyses, especially in light of the limited size of each spacer (see Huelsenbeck & Hillis, 1993) and the similarity in length, sequence variability, and G + C content of ITS-1 and ITS-2 in these plant groups. We therefore highly recommend that data from both ITS-1 and ITS-2 be combined for phylogenetic analyses of flowering plants unless strong incongruence be-

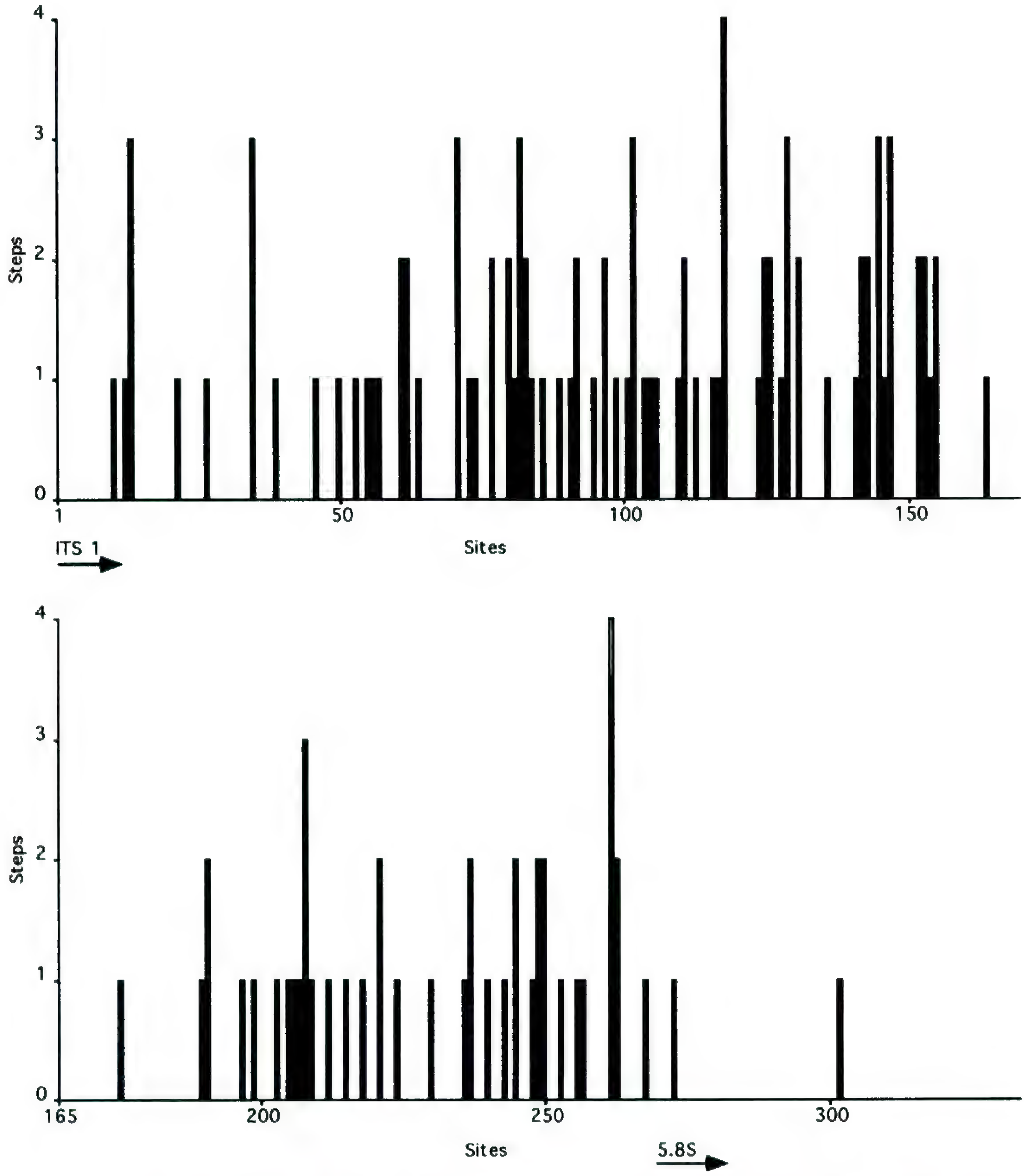


FIGURE 2 (pp. 256–257). Histogram of the number of changes at each aligned site of the ITS region in the 11 species of *Adenothamnus, Argyroxiphium, Dubautia, Madia, Raillardella, Raillardiopsis,* and *Wilkesia* (Asteraceae: Madiinae) discussed in Baldwin (1992). These changes were calculated across both minimum-length Fitch parsimony trees of these sequences using MacClade 3.0 (Maddison & Maddison, 1992). Numbers of changes at sites are the same in each of the two trees except at sites 261 and 532, for which the maximum numbers of changes are shown. Note interspersion of conserved and variable sites in both spacers and lack of variation in most of the 5.8S subunit. Aligned sequences are from Baldwin (1992), minus the five sites at which nucleotides were present only in outgroup species (which were not included in the McClade 3.0 analysis).

tween these spacers is indicated (see de Queiroz, 1993), such as by conflict between well-supported relationships in ITS-1 and ITS-2 trees. We have not observed such discordance between ITS-1 and ITS-2 data sets in angiosperms. To the contrary, these two spacers appear to be particularly good examples of "homogeneous" (Bull et al., 1993) sets of characters that have evolved similarly and are, therefore, best analyzed together for maximal phylogenetic resolution and support.

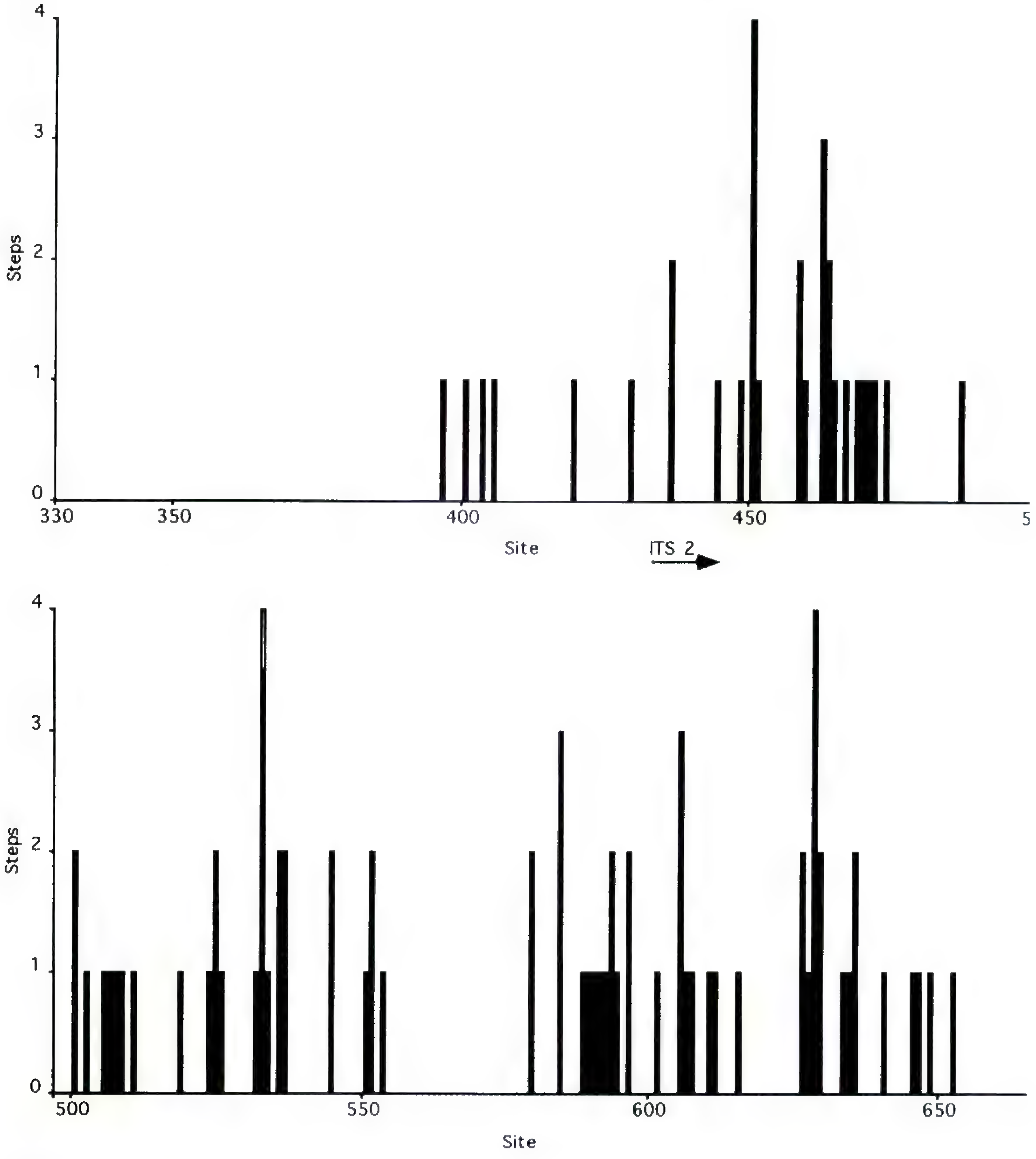


FIGURE 2. Continued.

G + C CONTENT VARIATION

Intraspecific G + C content in ITS-1 is roughly similar to that in ITS-2 in the same angiosperm sequences (Table 1), as seen in most other examined eukaryotes (Torres et al., 1990). This interspacer similarity probably reflects some degree of coevolution of ITS-1 and ITS-2 sequences, as suggested by evidence that both spacers are involved in maturation of large subunit rRNAs in *Saccharomyces cerevisiae* (van der Sande, 1992; van Nues et al., 1994). Overall ITS G + C content varies widely across angiosperm species, however, from ca. 50% in several groups to ca. 75% in *Oryza sativa* L. (Poaceae; Table 1). *Arceuthobium* (Viscaceae) falls outside this range, with unusually low ITS G + C content (ca. ≤36%; Nickrent et al., 1994; D. Nickrent, pers. comm.). Skewed G + C content may indicate a bias in substitution probabilities that could reduce detectability of homoplasy in such sequences compared to those with more equitable substitution expectations. A more practical concern about taxa with G + C rich ITS regions, however, is the difficulty of obtaining in-

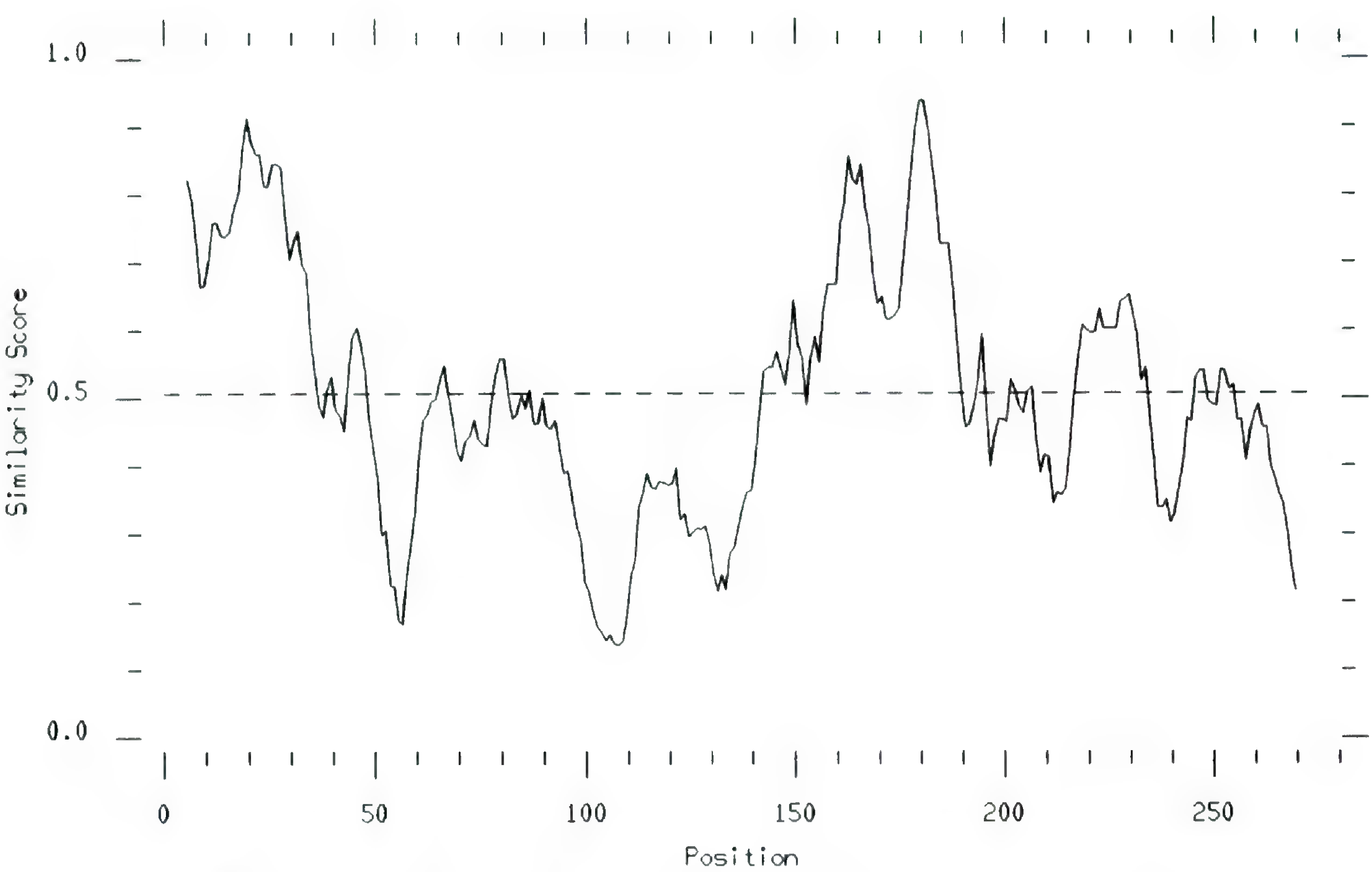


Figure 3 (pp. 258–259). Similarity of ITS sequences of 20 species (four per family) in Adoxaceae (*Viburnum*), Asteraceae (*Madia, Raillardiopsis*), Fabaceae (*Astragalus, Biserrula, Clianthus, Oxytropis*), Polemoniaceae (*Gilia, Ipomopsis, Loeseliastrum*), and Rosaceae (*Amelanchier, Cormus, Cydonia, Eriobotrya*) based on a PILEUP alignment (GCG, Genetics Computer Group, Madison, Wisconsin). Charted using PLOTSIMILARITY (GCG).—3A (this page). ITS-1.—3B (facing page). ITS-2.

terpretable DNA sequences because of the propensity for strong intrastrand Watson-Crick base pairing during sequencing reactions and sequencing-gel electrophoresis (see "Technical considerations for ITS sequencing").

INDELS: LIABILITIES AND OPPORTUNITIES

Most variation between angiosperm ITS sequences is attributable to point mutations rather than length mutations. Nevertheless, in contrast to most coding sequences, alignment of ITS sequences can be complicated by insertion/deletion (indel) mutations, which require insertion of gaps to preserve positional homologies of nucleotides. In our studies, sequence alignment required insertion of gaps in at least one sequence at only a minor to moderate proportion of sites, except in a family-wide analysis of Polemoniaceae that tested the outer limits of phylogenetic utility of the ITS region (Table 2). Placement of gaps in most indel regions proved unambiguous because of flanking conserved sites. In general, interspersion of conserved and variable positions through both spacers promotes sequence alignment in the ITS region (Fig. 2).

In most groups at least one region in both ITS-1 and ITS-2 appears to have experienced relaxed evolutionary constraint on length. Unambiguous alignment of sequences in such segments may be impossible because of independent and overlapping length mutations that have occurred in different taxa. Such regions have required, however, elimination of only a small proportion of total nucleotide positions from our phylogenetic analyses. Bruns et al. (1992) provided helpful examples of how character states within indel regions can be recoded to maximize extraction of phylogenetic information without overweighting characters or using questionably aligned sequence data.

Although alignments may be problematical in some ITS segments, the occurrence of indels can be seen as an opportunity rather than a liability. For example, in *Calycadenia* all but one of several potentially informative indels mapped without homoplasy onto trees based only on nucleotide site-substitutions (Baldwin, 1993a). Two of these indels provided phylogenetic markers for otherwise weakly supported lineages. Other angiosperm ITS studies have shown a similar lack of homoplasy in indels (e.g., Baum et al., 1994; Donoghue & Baldwin, 1993, unpublished; Baldwin & Robichaux, 1995). Based on these findings, it may be desirable to take advantage of information provided by unambigu-

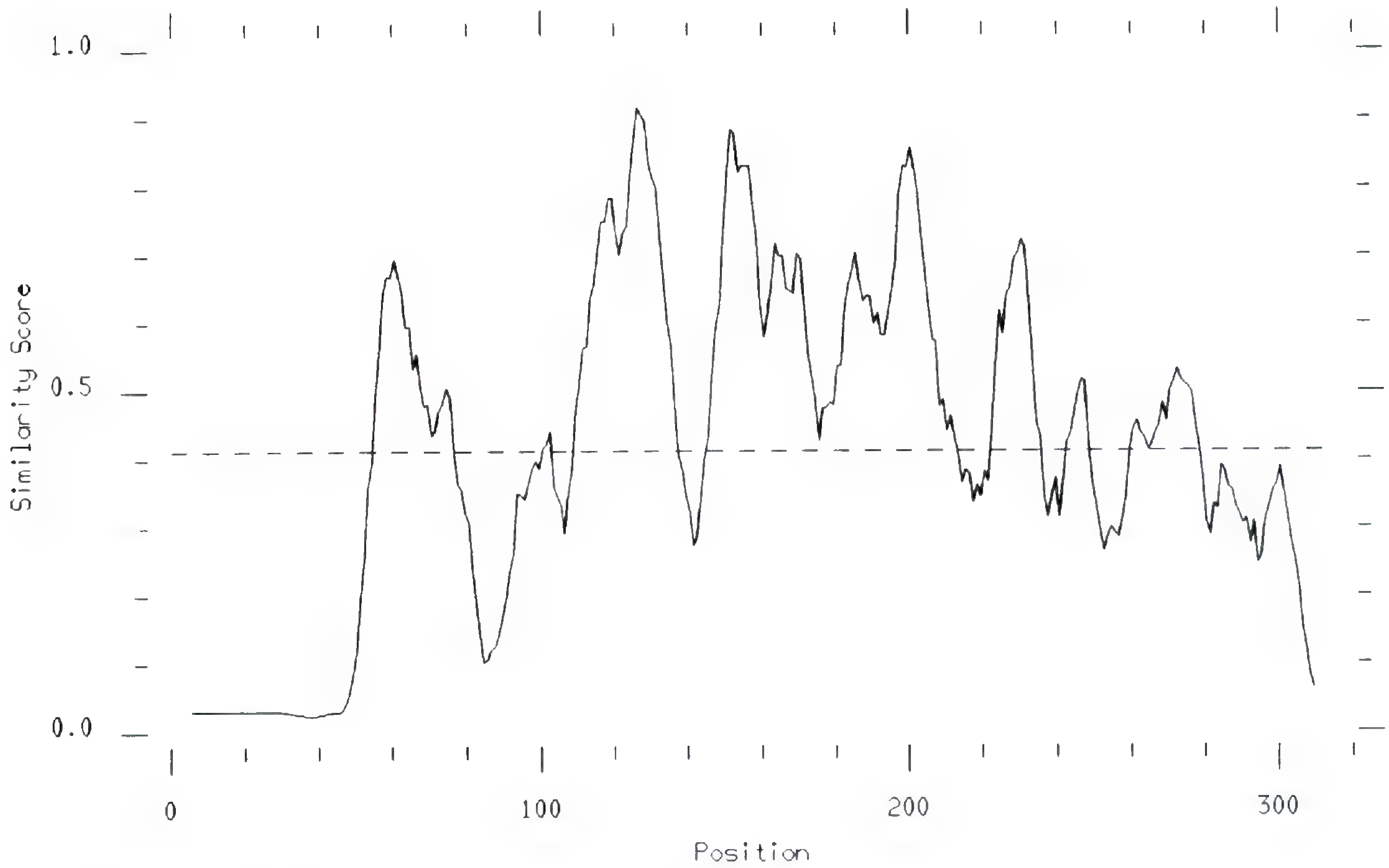


FIGURE 3. Continued.

ously placed indels rather than removing them entirely from phylogenetic analyses.

There are several ways of treating indel characters (see Swofford & Olsen, 1990; Bruns et al., 1992; Wojciechowski et al., 1993). For example, gaps introduced in indel regions can be treated as missing data or as a fifth character state. Treatment of gaps can be more problematical, however, in indels that span multiple nucleotide sites, even if gap placement is unambiguous (Wojciechowski et al., 1993). Overscoring of such indels is, of course, ensured if gaps are treated as a fifth character state at more than one nucleotide position. Alternatively, the indel may be recoded as an additional presence/absence character, with sequence gaps treated as missing data (Baum et al., 1994; Donoghue & Baldwin, 1993, unpublished). This last option, with or without recoding of the indel, requires that state-assignments to missing-data characters be examined following phylogenetic analysis to assess any effects on the resultant trees (assuming that potential phylogenetic information, aside from the indel, is present in the indel region). Although indels can introduce complications to phylogenetic analysis, the effects of different indel treatments (including exclusion) on resultant ITS tree topologies, where examined in angiosperms, have been minimal or absent (Wojciechowski et al., 1993; Baldwin, 1993a, unpublished; Baldwin & Robichaux, 1995; Baum et al., 1994; Donoghue & Baldwin, 1993, unpublished).

CONSIDERATION OF SECONDARY STRUCTURE

Alignments of ITS-1 sequences across five plant families show insufficient retention of sequence similarity to allow unambiguous alignment except near the highly conserved 18S end of the spacer, and, to a lesser extent, near the center of the spacer (Fig. 3A). Reconstructions of ITS-1 secondary structures across these families, using FOLD in GCG (Genetics Computer Group, Madison, Wisconsin), also exhibit a lack of similarity. In contrast to ITS-1, ITS-2 displays somewhat more sequence similarity across families in the central region of the spacer (Fig. 3B).

Reconstructed secondary structures of ITS-2 sequences in species of *Calycadenia* (Asteraceae), *Gilia* (Polemoniaceae), and *Viburnum* (Adoxaceae) (using FOLD in GCG) show great similarity, with a common three-stem/loop structure, like that of *C. oppositifolia* (E. Greene) E. Greene (Fig. 4). These conformational similarities may reflect stronger structural constraints on ITS-2 than on ITS-1 in these taxa (all in Asteridae sensu lato), which also show less sequence variation in ITS-2 than in ITS-1 (Table 2). It should be noted, however, that only minor differences in free energy exist between

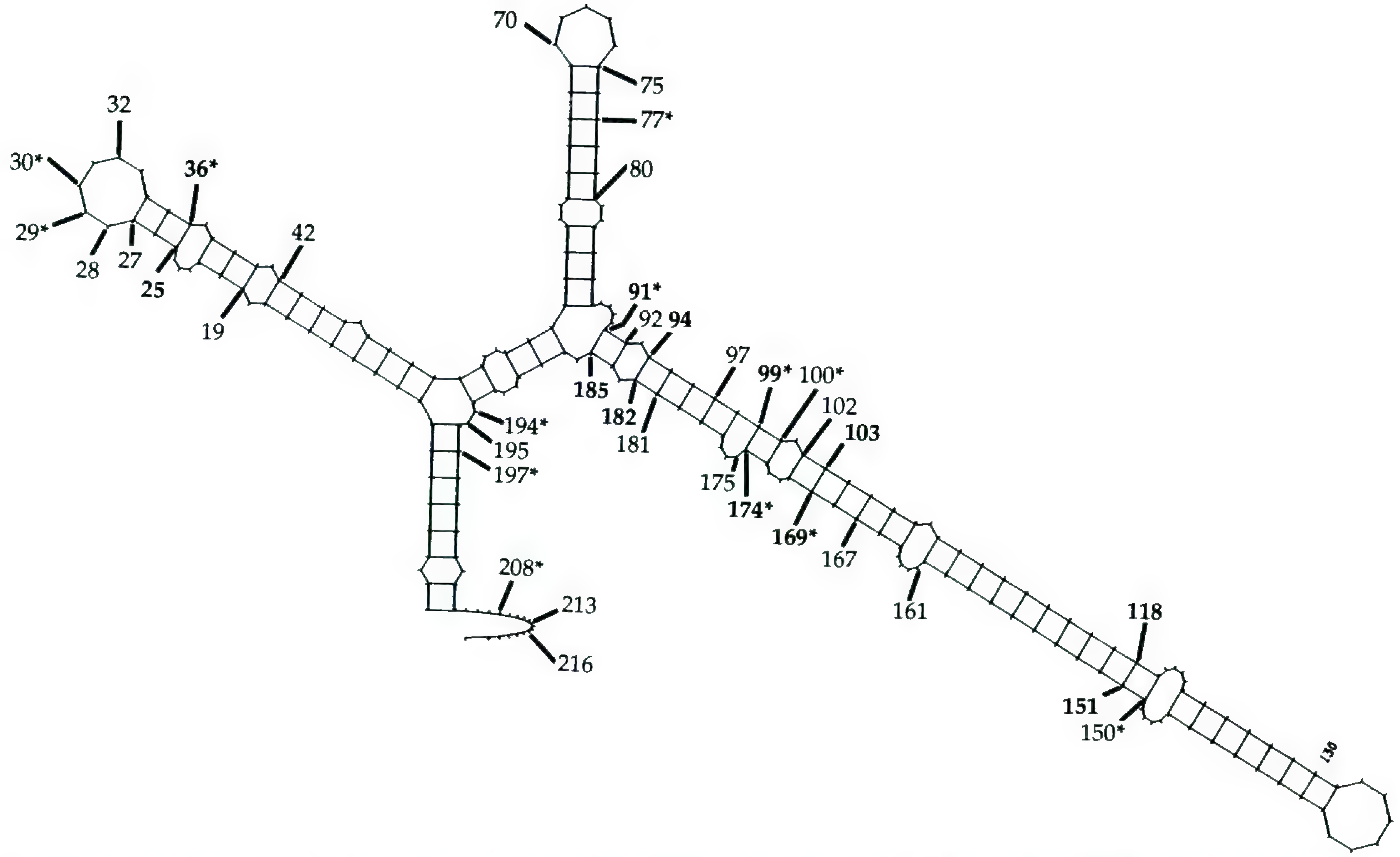


FIGURE 4. Hypothetical secondary structure of ITS-2 RNA of *Calycadenia oppositifolia* (Asteraceae) based on a minimum free-energy reconstruction using FOLD and SQUIGGLES in GCG (Genetics Computer Group, Madison, Wisconsin). Numbered sites marked by lines are positions where nucleotide variation was detected among species of *Calycadenia*. Asterisks indicate positions at which more than one evolutionary change possibly occurred in *Calycadenia*, based on MacClade 3.0 (Maddison & Maddison, 1992) character-evolution reconstructions on one of the 11 maximally parsimonious trees found by Baldwin (1993a). Bold numbers indicate variable sites in direct opposition. All changes at these directly opposing variable sites occurred along different branches of the phylogeny and therefore appear to be evolutionarily independent, based on this provisional secondary structure.

the most parsimonious structures reconstructed by FOLD and other conformations that can be radically different in shape. In addition, minimum free-energy ITS-2 conformations in some species of *Calycadenia*, *Gilia*, and *Viburnum* were quite different from that depicted in Figure 4. The ITS-2 reconstruction presented here (Fig. 4) should, therefore, be interpreted as a highly provisional estimate. Perhaps as algorithms for reconstructing secondary structures become more sophisticated it will be possible to use such conformational features as additional characters in phylogenetic analyses, much as complex morphological characters are used presently.

Potential non-independence of characters complicates phylogenetic analysis of DNA sequences, such as those of the ITS region, that produce transcripts with seemingly functional secondary structures. Mutations at ITS positions involved in stem formation (via intrastrand nrRNA pairing) may necessitate compensatory mutations at directly opposing sites to maintain structural integrity and proper functioning of the molecule. Differing estimates of the prevalence of such linked changes in nrDNA have led to various character-weighting recommendations, ranging from 50% weighting of all stem characters relative to loop sites in 5S and 5.8S rDNA (Wheeler & Honeycutt, 1988) to $\geq$80% weighting of stem sites compared to loop sites in large subunit (26S) rDNA (Dixon & Hillis, 1993). Given that Dixon & Hillis (1993) found $<$40% as many compensatory changes in 26S rDNA as expected under a model of 100% compensation in stem regions, the extent of such non-independence in ITS sequences needs to be determined empirically.

A preliminary search for compensatory changes in ITS-2 sequences of *Calycadenia* failed to reveal any such mutations. The evolutionary history of ITS-2 sequences in *Calycadenia* was first inferred from examining all possible character changes along one of the 11 maximally parsimonious ITS trees (Baldwin, 1993a) that appeared most compatible with cytology and morphology. These mutations, reconstructed using MacClade 3.0 (Maddison & Maddison, 1992), were mapped onto minimum free-energy ITS-2 structures of most *Calycadenia* species, one of which is shown in Figure 4. This approach revealed two to six pairs of changes that occurred at directly opposing positions along stems. Opposing mutations of a pair, however, represented independent events that occurred in different lineages, i.e., the changes were apparently unlinked evolutionarily. Simultaneous changes in a single lineage (along the same branch of the tree) did occur within two to three stem regions where clusters of changes were found. None of these mutations were directly opposing changes, but their close proximity may signal cryptic non-independence. Slight readjustments of intrastrand RNA pairing through slippage could obscure evidence of compensation in such instances. This possibility needs to be explored by considering secondary structures of ancestral sequences that existed at the time of each change, i.e., structures inferred for each node in the tree. In addition, improved reconstructions of ITS secondary structure may reveal evidence of more compensatory change. Alternatively, selection for compensatory mutations in this spacer may be weaker than in the nrDNA subunits. If so, concerns about non-independence of ITS mutations would be alleviated.

Limits of Phylogenetic Utility

Taxonomic Levels of Utility

Available data indicate that ITS sequences are phylogenetically useful at various intrafamilial levels in angiosperms, depending on the lineage, but are unlikely to retain sufficient evolutionary signal or alignability for examining relationships among species in different plant families (but see Hershkovitz & Zimmer, 1994). Of course, this generalization holds only to the extent that family rank signifies an ancient origin, which is certainly not true in all cases. In addition, low levels of ITS variation in some ancient plant groups (see below) raise the possibility that the ITS region may prove useful for assessing relationships between old families that have experienced exceptionally low rates of spacer evolution. The arbitrariness of taxonomic ranks is a major impediment to any general statements about taxonomic limits on the phylogenetic utility of ITS sequences.

Divergence between ITS sequences has apparently been sufficient to render family-wide phylogenetic studies untenable in Asteraceae (Baldwin, 1991, 1992; Kim & Jansen, 1994), Fabaceae (Sanderson & Wojciechowski, unpublished), Rosaceae (Campbell, Donoghue, Baldwin & Wojciechowski, unpublished), Saxifragaceae (D. Soltis & Kuzoff, in press), Viscaceae (D. Nickrent, pers. comm.), and, to a lesser extent, Polemoniaceae (Porter, 1993a, b). In these groups, ITS sequences have proven most valuable for examining relationships within genera and among the more closely related genera. ITS sequences do, however, appear suitably conserved for family-wide phylogenetic studies in Fouquieriaceae (Schultheis & Baldwin,

1994), Nothofagaceae (Manos, 1993), and Winteraceae (Suh et al., 1993).

Within species, ITS sequences show some promise for investigations of relationships among allopatric or disjunct populations. For example, up to 3.7% ITS sequence divergence was found between individuals from conspecific, allopatric populations in *Calycadenia* (Baldwin, 1993a). Some of this variation was potentially informative for resolving relationships among subspecies.

In view of the relatively large number of DNA sequences identified for higher-level, as opposed to intrageneric or other lower-level, phylogenetic analyses in flowering plants, we feel that ITS sequences present special opportunities for evolutionary studies. The small number of nucleotide positions available for analysis in both spacers is, in several groups, compensated for by the high levels of variation found in ITS-1 and ITS-2. In fact, in several groups ITS sequences are much more variable than cpDNA from the same total DNA accessions, as estimated from the plastome as a whole based on cpDNA restriction site data [e.g., Madiinae (Baldwin, 1989, 1992), *Astragalus* (Sanderson & Doyle, 1993; Wojciechowski et al., 1993), Rudbeckiinae (Urbatsch & Jansen, 1992; Urbatsch & Baldwin, 1993, unpublished), and *Viburnum* (Donoghue & Sytsma, 1993; Donoghue & Baldwin, 1993, unpublished)].

LIFE HISTORY AND EVOLUTIONARY RATES

ITS sequences appear to have evolved more slowly in some ancient woody groups than in herbaceous, primarily annual groups of comparatively recent origin. Similar levels of ITS sequence divergence were found between taxa in lineages that diverged in the early Tertiary or Cretaceous, e.g., sections of *Viburnum* (Donoghue & Baldwin, 1993, unpublished), genera of Winteraceae (Suh et al., 1993), or clades within *Nothofagus* (Manos, 1993), and between taxa in herbaceous lineages that presumably diverged in the Pliocene/Pleistocene (e.g., genera of Madiinae; Baldwin, 1992).

Such correlations between plant life-form and apparent rates of molecular evolution have been noted from cpDNA data as well (e.g., Schilling & Jansen, 1989; Wilson et al., 1990; Gaut et al., 1992, 1993; Brunsfeld et al., 1994), although the basis for this pattern is unclear. A generation-time explanation for differences in molecular evolutionary rates (with longer generation times resulting in slower molecular evolution), although roughly consistent with observed patterns and perhaps compelling for some animals, is problematical in plants, wherein open development might allow fixation of mutations in vegetative meristems as readily as in reproductive cells that arise from such meristems (Klekowski, 1988). Generational turnover is also a more complex issue in plants than in animals because of the potentially stabilizing effect of seed banks and clonal reproduction on evolutionary rates in herbs. This is an area that needs more theoretical and empirical attention by plant molecular evolutionists.

IMPORTANCE OF PHYLOGENETIC RESULTS FROM THE ITS REGION

Independent lines of phylogenetic evidence can be of great value for identifying and resolving conflicting hypotheses of relationship and for providing enhanced resolution within lineages. The ITS region has proven repeatedly to be of such benefit in angiosperm studies. Below, we provide examples that demonstrate how ITS characters have aided understanding of plant phylogeny by providing (1) corroboration of unexpected findings, (2) resolution of conflict between data sets, (3) improved resolution of species relationships, or (4) direct resolution of reticulate evolution. These four categories are, to some extent, overlapping and some of the results discussed under one heading are actually exemplary of two or more of these types of findings.

CORROBORATION OF UNEXPECTED FINDINGS

A cpDNA restriction site study of the Madiinae (Compositae) indicated a previously unpredicted relationship between the Hawaiian silversword alliance and a subgroup of California tarweeds (Baldwin, 1989, 1992; Baldwin et al., 1991) (Fig. 5A). The cpDNA trees strongly indicate that the Hawaiian lineage (*Argyroxiphium*, *Dubautia*, and *Wilkesia*) is nested within Californian *Madia*/*Raillardiopsis*. An ITS phylogeny of these same species provides robust corroboration of this finding (Baldwin, 1992) (Fig. 5B), thereby reinforcing and extending Carlquist's (1959) hypothesis that the Hawaiian silversword alliance originated from American tarweeds.

An ITS phylogeny of subfamily Maloideae (Rosaceae) places *Vauquelinia*, a capsular-fruited species usually referred to tribe Quillajeae of Spiraeoideae, within the pome-fruited Maloideae (Fig. 6) (Campbell et al., in press). This result supports the floral morphological findings of Sterling (1966) and the conclusion of Goldblatt (1976) based on chromosomal and morphological evidence. cpDNA *rbc*L sequences place *Vauquelinia* and other Quillajeae

at the base of Maloideae (Morgan et al., 1994), in near agreement with the ITS results.

An ITS phylogeny of Polemoniaceae (Fig. 7) (Porter, 1993a, b) provides several examples of unexpected relationships that corroborate recent phylogenetic findings based on cpDNA *mat*K sequences (Steele & Vilgalys, 1994; Johnson & Soltis, 1995). For example, in both the ITS and cpDNA trees, the spiny-leaved genus *Navarettia* is placed within a clade that includes species of *Allophyllum*, *Collomia*, and *Gilia*, and outside a lineage that includes other spiny-bracted genera, such as *Eriastrum*, *Langloisia*, and *Loeseliastrum*, with which *Navarettia* has been traditionally associated. In addition, *Phlox* appears closely related to *Leptodactylon* and *Linanthus* in both the ITS and cpDNA trees, a result previously unpredicted but supported by the unusual occurrence of opposite leaves in these genera, a potential morphological synapomorphy.

An ITS phylogeny of Galegeae (Fabaceae) (Sanderson & Wojciechowski, unpublished; Sanderson & Liston, in press) corroborates cpDNA evidence (Sanderson & Liston, in press) that this tribe and *Astragalus* are paraphyletic. Another unanticipated finding from both lines of evidence is the existence of a strongly supported core clade of genera that comprises all of subtribe Coluteinae and the astragaloid part of subtribe Astragalinae. ITS sequences of members of this core clade show high similarity, but are highly divergent from those of other members of Galegaeae.

RESOLUTION OF CONFLICT BETWEEN DATA SETS

A striking example of conflict resolution by ITS data involves *Viburnum* (Adoxaceae). A morphological phylogeny of *Viburnum* (Fig. 8A) suggested that the purple-fruited species of section *Oreinotinus* from the New World and some members of section *Odontotinus* are the sister group of a predominantly Asian, red-fruited clade of section *Opulus* and the other members of section *Odontotinus* (Donoghue, 1983). A cpDNA phylogeny (Fig. 8B), however, indicated that one of the purple-fruited species, *V. acerifolium* L., is more closely related to certain Asian, red-fruited species than it is to other New World taxa (Donoghue & Sytsma, 1993, unpublished). Initially, these results raised concern that the cpDNA trees deviated from the species tree because of introgression or cpDNA lineage sorting. An ITS phylogeny (Fig. 8C), however, corroborates the cpDNA placement of *V. acerifolium* with the Asian, red-fruited group (Donoghue & Baldwin, 1993, unpublished). In this case, reexamination of morphology indicates that several characters that were not included in the previous analysis, including subtle differences in endocarp shape (Donoghue & Strauss, 1988), support the molecular results. Congruence among ITS, cpDNA, and endocarp data strongly indicates that the evolution of fruit color has been more complex than implied by the original morphological analysis.

In the Hawaiian silversword alliance, cytogenetic (Carr & Kyhos, 1986, pers. comm.) and cpDNA data (Baldwin, 1989; Baldwin et al., 1990) conflict about relationships of several species of *Dubautia* from Kaua'i that possess either the D1 or D3 chromosomal arrangements. The cpDNA trees strongly support species groupings that indicate multiple origins of one or both of these chromosomal arrangements (Fig. 9A). The ITS data, however, robustly support monophyly of the D1 species and paraphyly of the D3 species, relationships compatible with single origins of both genomic arrangements (Fig. 9B) (Baldwin, unpublished). Further, ITS data, unlike the cpDNA results, are highly congruent with an ordered evolutionary series of three chromosomal arrangements (including the two in question), with adjacent members of this series separated by single reciprocal translocations (see Fig. 9). These data, together with other evidence, indicate that the cpDNA results are, in part, incongruent with the nuclear DNA component of species relationships, probably as a result of introgressive hybridization (Baldwin, unpublished).

In the *Heuchera* group (Saxifragaceae), cpDNA trees strongly suggest that northern and southern populations of *Tellima grandiflora* (Pursh.) Dougl. are not closely related, a result attributed to introgressive hybridization between a species of *Mitella* and *T. grandiflora* that led to chloroplast capture of *Mitella* cpDNA by *Tellima* (Soltis et al., 1991a, b). ITS trees of the *Heuchera* group strengthen this introgression hypothesis by strongly corroborating morphological and isozymic evidence that the northern and southern populations of *T. grandiflora* are conspecific (Soltis & Kuzoff, in press).

IMPROVED RESOLUTION OF SPECIES RELATIONSHIPS

An ITS phylogeny of *Astragalus* (Fig. 10) (Wojciechowski et al., 1993) provides strong support for a lineage previously unpredicted from morphological evidence but congruent with biogeographic and cytological data. All of the aneuploid species of the New World sampled, with chromosome numbers ranging from $n = 11$ to $n = 15$, form a strongly supported monophyletic group in

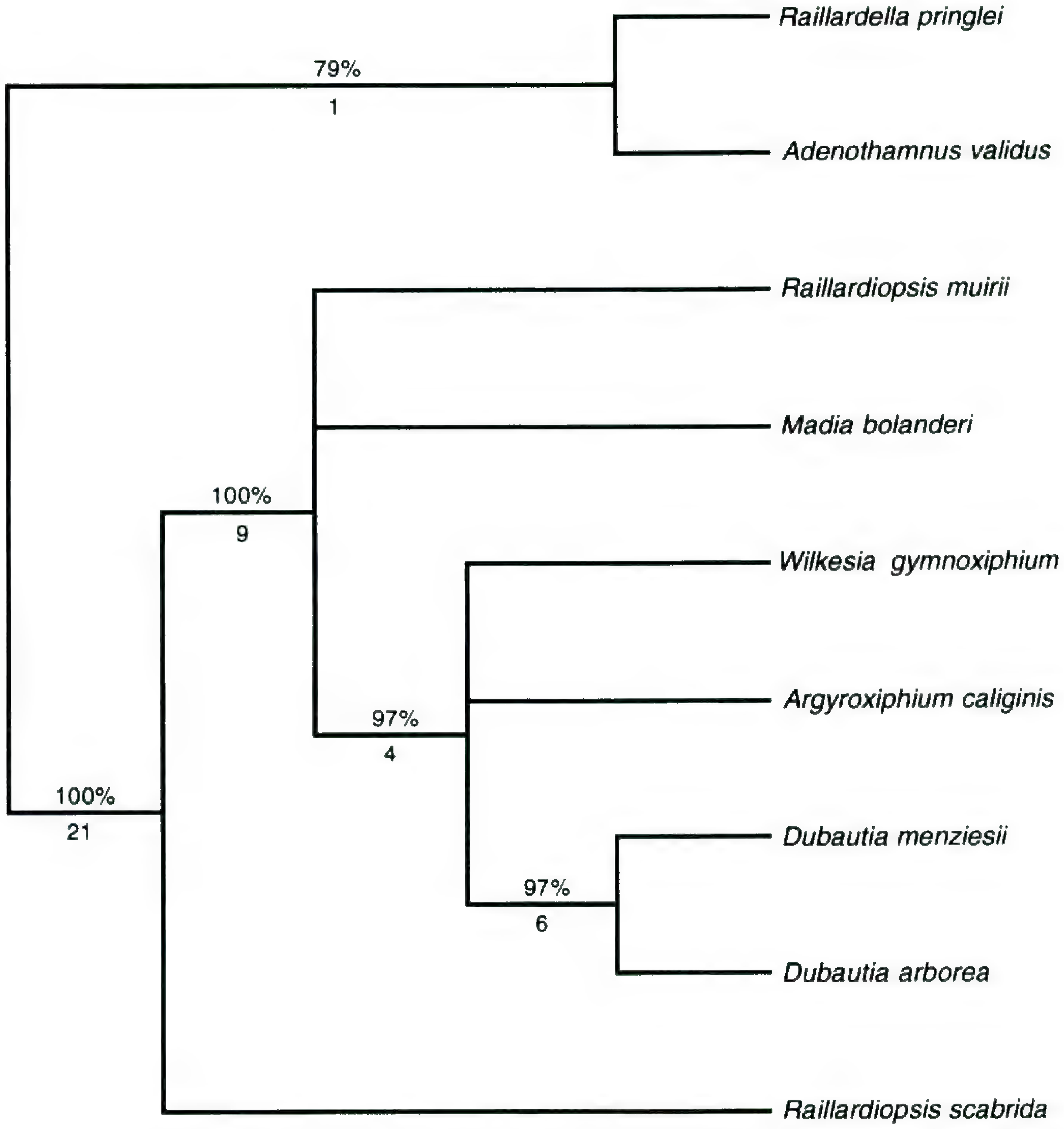


FIGURE 5 (pp. 264–265). Strict consenses of maximally parsimonious DNA trees of Californian and Hawaiian Madiinae (Asteraceae).—5A (this page). cpDNA restriction site tree (reprinted with permission from Baldwin, 1992).—5B (facing page). ITS sequence tree (reprinted with permission from Baldwin, 1992). Bootstrap percentages are presented above the branches. Decay index values appear below the branches. Asterisks denote outgroup species outside Madiinae. Monophyly of the large Hawaiian silversword alliance (*Argyroxiphium*, *Dubautia*, and *Wilkesia*) is strongly supported by more inclusive parsimony analyses of cpDNA (Baldwin, 1989; Baldwin et al., 1990, 1991) and ITS (Baldwin & Robichaux, 1995) data. Note that the Hawaiian species are nested within *Madia/Raillardiopsis* in trees A and B.

the ITS trees despite the lack of a single known morphological character that could thus unite these taxa. Monophyly of this aneuploid species assemblage is congruent with cpDNA restriction site data from a less extensive species sampling (Sanderson & Doyle, 1993). This large, morphologically cryptic lineage is of sufficient biogeographic and cytogenetic importance to warrant taxonomic recognition (Wojciechowski et al., 1993).

In the Hawaiian silversword alliance, cpDNA restriction site studies failed to discern a single restriction site mutation that could potentially unite the species of *Argyroxiphium*, the true silverswords and greenswords, as a monophyletic group (Baldwin, 1989; Baldwin et al., 1990). In sharp contrast, one of the two longest branches in the ITS tree supports monophyly of *Argyroxiphium* (Baldwin & Robichaux, 1995; Baldwin, unpub-

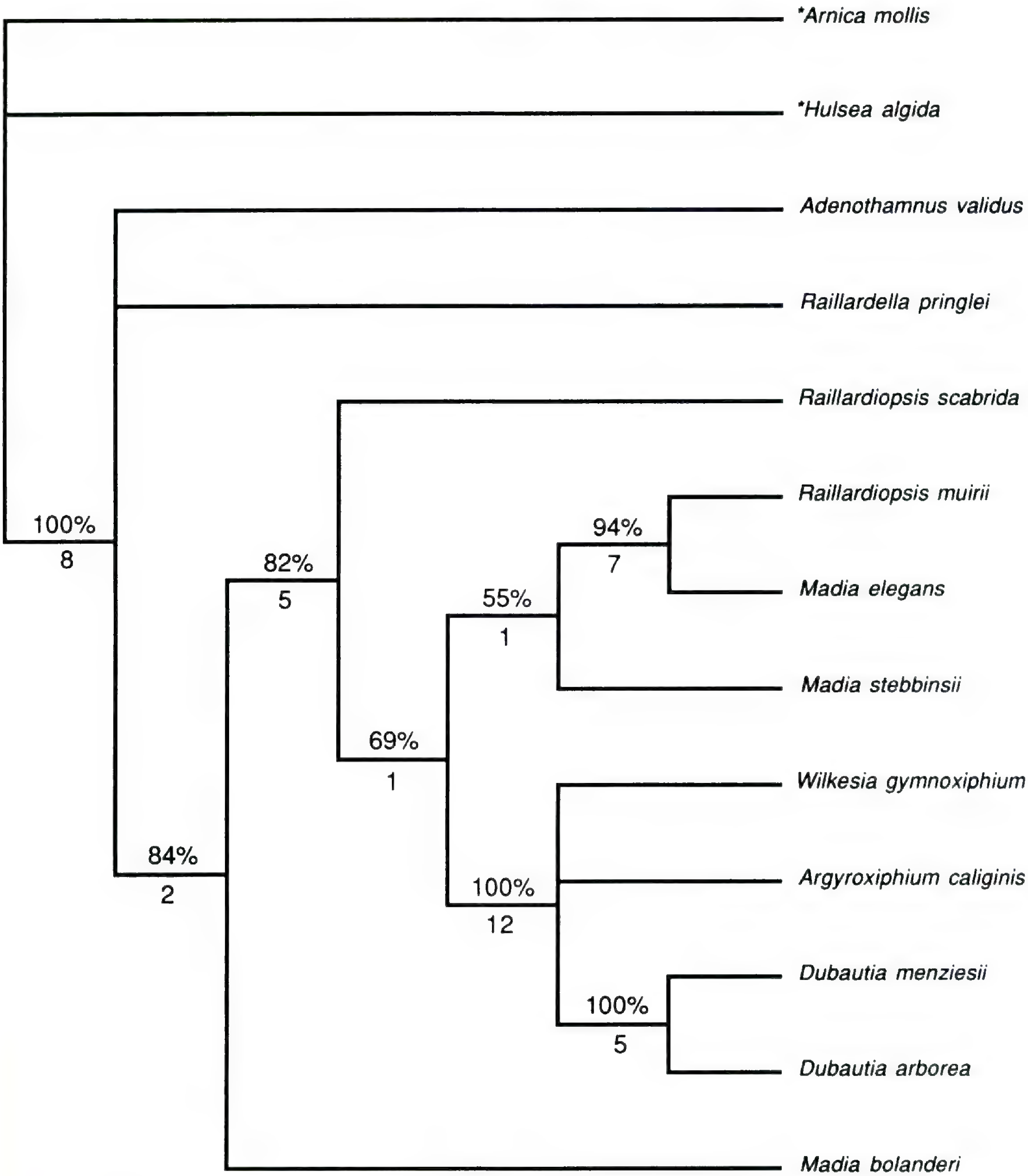


FIGURE 5. Continued.

lished). This provides a striking example of how inequity in relative rates of ITS as opposed to cpDNA evolution can affect phylogenetic resolution.

DIRECT EVIDENCE OF RETICULATE EVOLUTION

Unlike cpDNA, nrDNA data can provide direct evidence of reticulate evolution if concerted evolution fails to act across repeat units contributed by different parental species. Such lack of sequence homogenization may occur if, for example, (1) the hybridization event was recent, (2) nrDNA repeats are at different loci in the parental taxa (e.g., on different chromosomes) and interlocus gene conversion is inoperative in their hybrid, or (3) the hybrid is asexual. Parentage of suspected early generation hybrids may be resolved simply by screening for presence or absence of restriction sites diagnostic for ITS sequences of each of the

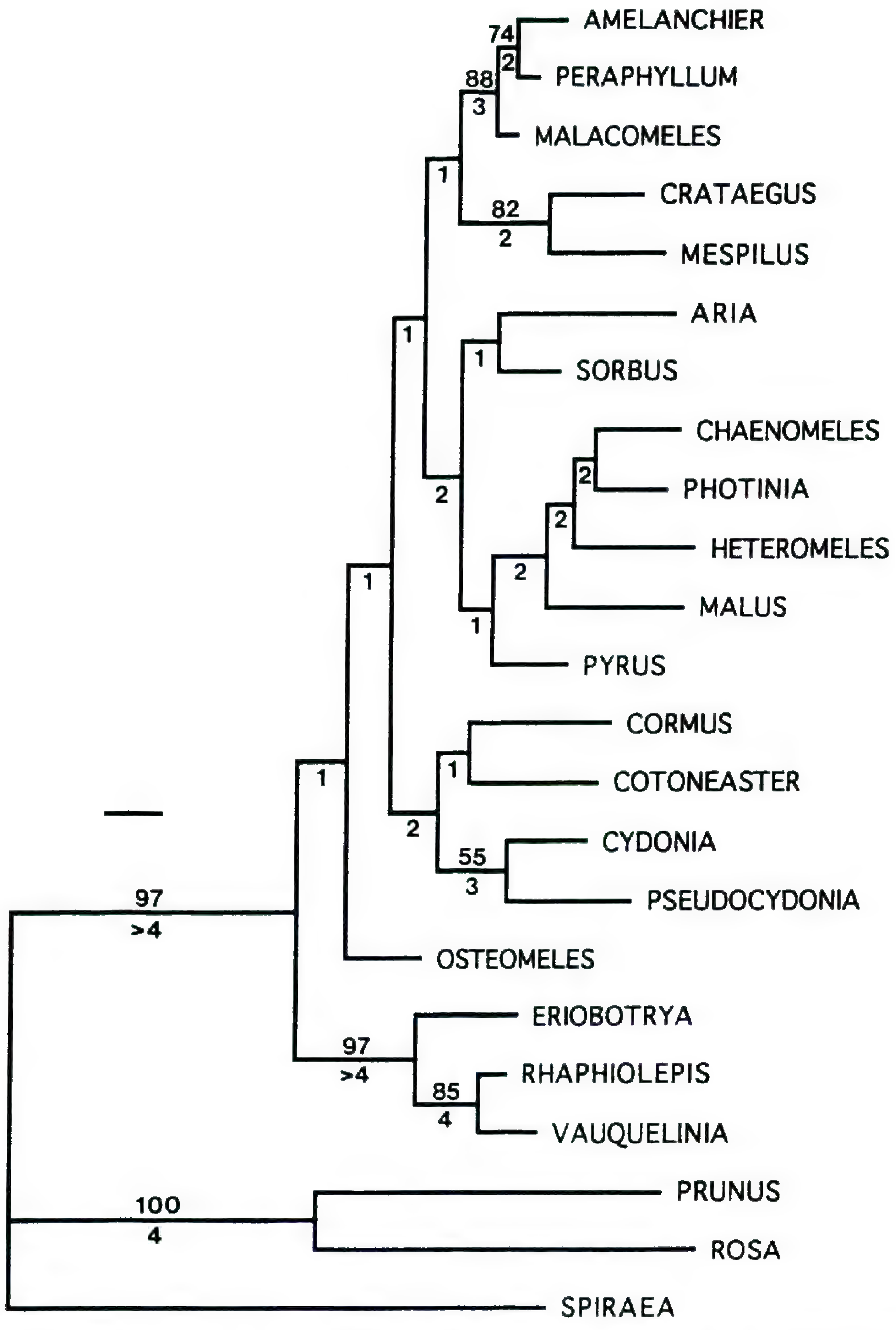


FIGURE 6. Phylogram of the single most-parsimonious tree based on ITS and partial 5.8S rDNA sequence variation in Maloideae (Rosaceae) (Campbell et al., 1993a; in press). The tree is rooted between *Prunus*, *Rosa*, and *Spiraea* and the 19 Maloideae genera plus *Vacquelinia*. Branch lengths are based on accelerated transformation (ACCTRAN) of characters. The separate bar indicates the branch length equivalent to 10 substitutions. Numbers above branches indicate bootstrap percentages for clades found in both this tree and the bootstrap majority rule consensus tree. Numbers below branches are decay index values. Note the well-supported position of *Vauquelinia* (Quillajeae) within Maloideae.

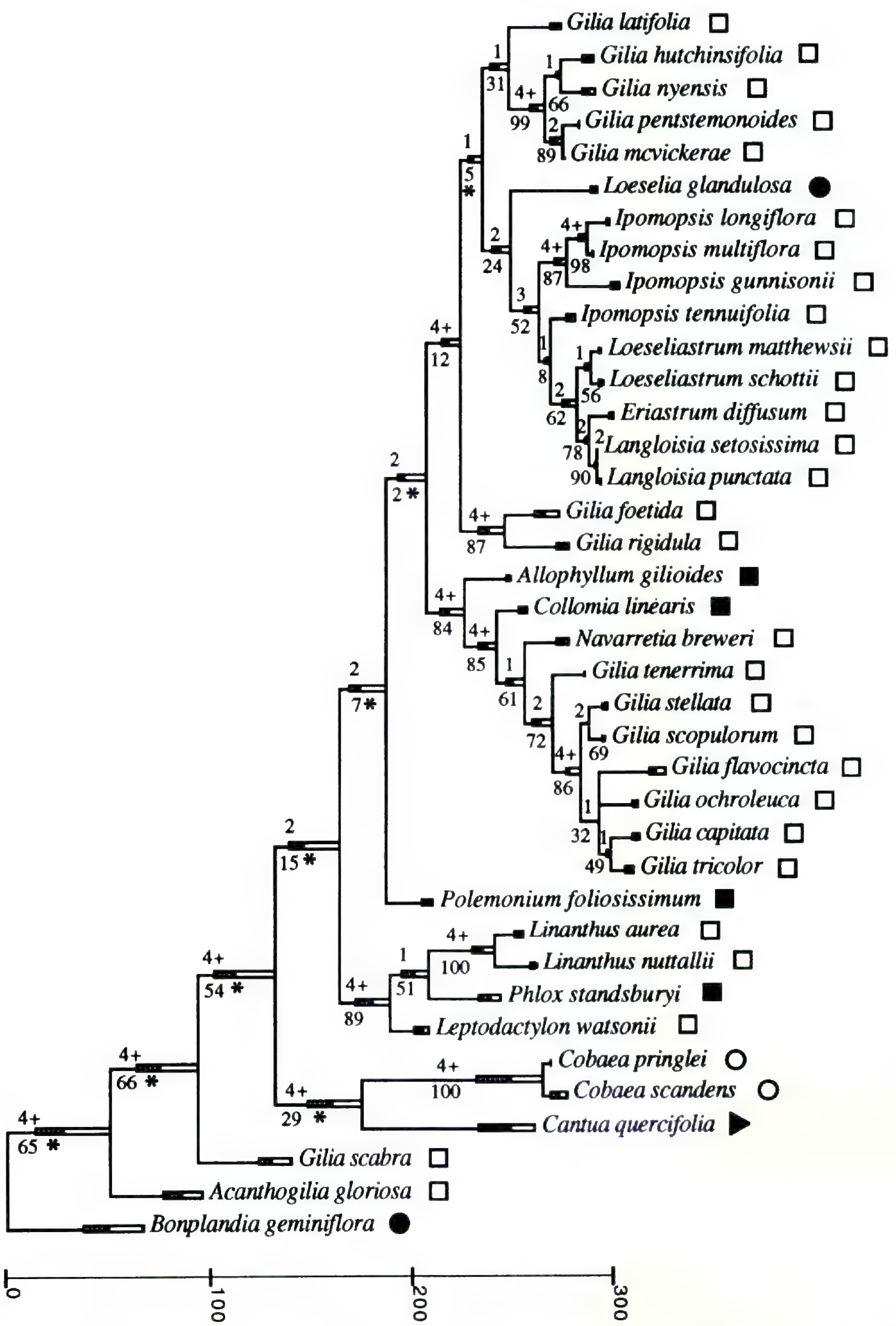
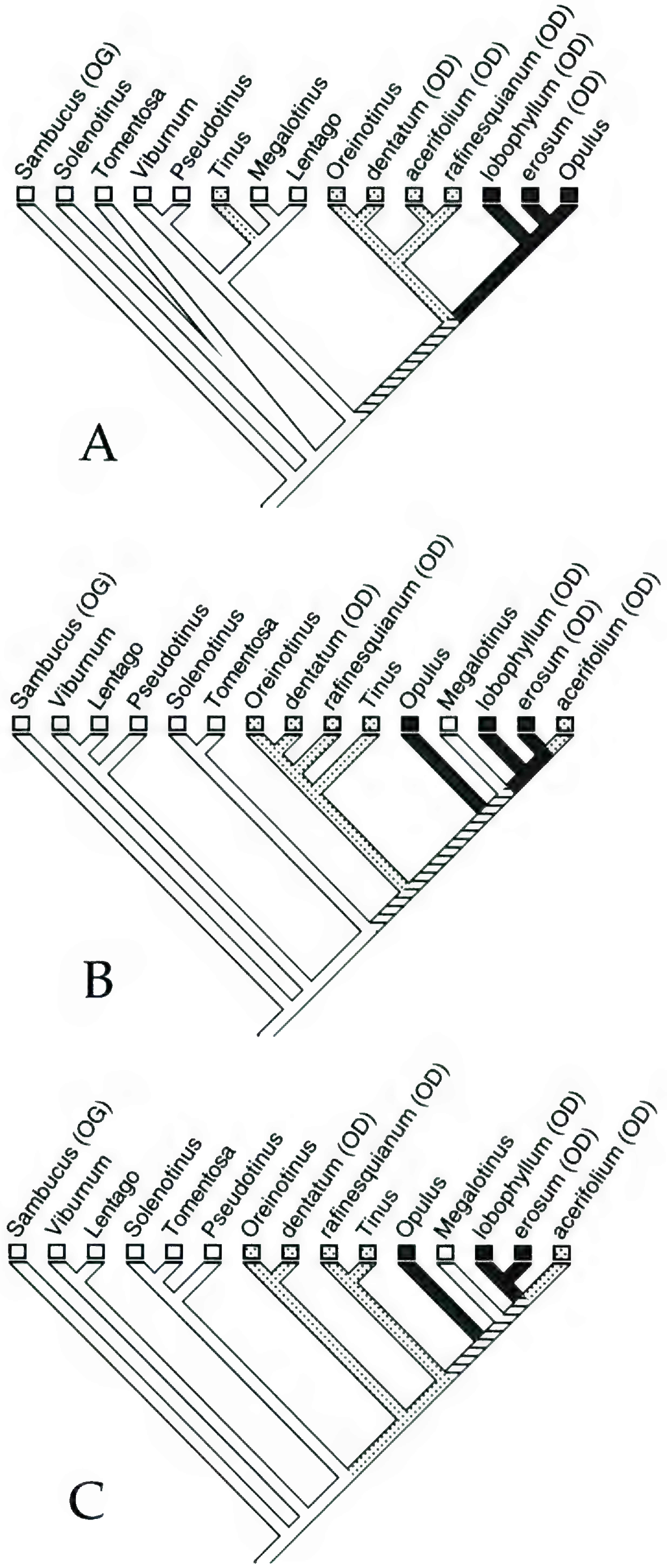


FIGURE 7. Strict consensus of maximally parsimonious trees based on ITS sequences of Polemoniaceae (Porter, 1993a, b). *Fouquieria* (not shown) is the outgroup. Branch lengths were calculated using the minimum-average-maximum reconstruction procedure of MacClade 3.0 (Maddison & Maddison, 1992), with the internal nodes placed at the maximum value. Bootstrap percentages are given below the branches. Decay index values appear above the branches. Nodes for which phylogenetic estimates are considered unreliable, based on jackknifing of taxa, and g_1 values of tree length distributions are identified with an asterisk. Tribal placement (Grant, 1959) of each species is noted as follows: open square = *Gilia* tribe, closed square = *Polemonium* tribe, open circle = *Cobaea* tribe, closed circle = *Bonplandia* tribe, closed triangle = *Cantua* tribe. The close relationships of (1) *Navarretia* to *Allophyllum, Collomia,* and part of *Gilia* and (2) *Leptodactylon, Linanthus,* and *Phlox* were unanticipated given the current tribal classification, but are supported by new cpDNA evidence (Steele & Vilgalys, 1994; Johnson & Soltis, 1995).


Sambucus (OG)
Solenotinus
Tomentosa
Viburnum
Pseudotinus
Tinus
Megalotinus
Lentago
Oreinotinus
dentatum (OD)
acerifolium (OD)
rafinesquianum (OD)
lobophyllum (OD)
erosum (OD)
Opulus
A
Sambucus (OG)
Viburnum
Lentago
Pseudotinus
Solenotinus
Tomentosa
Oreinotinus
dentatum (OD)
rafinesquianum (OD)
Tinus
Opulus
Megalotinus
lobophyllum (OD)
erosum (OD)
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Solenotinus
Tomentosa
Pseudotinus
Oreinotinus
dentatum (OD)
rafinesquianum (OD)
Tinus
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Megalotinus
lobophyllum (OD)
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presumed parental species. In such cases, additivity for the parental restriction patterns can provide excellent evidence of hybridity. Resolution of ancient hybridization, however, requires more detailed characterization of ITS variation, e.g., by sequencing of ITS clones.

A striking example of maintenance of parental ITS sequences in a presumed hybrid has been found in *Amelanchier* (Rosaceae) (Campbell et al., 1993b). Direct ITS sequences of *A.* × *neglecta* (Eggelst.) Eggelst. show additivity of nucleotide states at each site where the presumed parental species, *A. bartramiana* (Tausch) Roemer and *A. laevis* Wieg. (Weber & Campbell, 1989), differ. These data support the hypothesis that *A. bartramiana* and *A. laevis* are the parental species of *A.* × *neglecta*, although sequences from clones of the divergent repeat types in this apomict are being obtained to confirm this reconstruction.

Kim & Jansen (1994) detected a similar phenomenon by direct ITS sequencing of pooled PCR products from hexaploid *Krigia montana* (Michaux) Nutt. (Compositae). Previous cpDNA and nrDNA restriction site data (Kim & Jansen, 1992; Kim & Mabry, 1991) indicated that diploid *K. biflora* Blake and tetraploid *K. montana* were the maternal and paternal parents, respectively, of allohexaploid *K. montana*. Direct ITS sequences of these taxa revealed additivity of the nucleotide states that differentiate *K. biflora* and tetraploid *K. montana* in hexaploid *K. montana*. As in the *Amelanchier* study, cloning of ITS repeat-types will be necessary to determine exact sequences of these repeat types and to detect any possible recombinants.

Fixation of maternal and paternal repeat types in different populations of a hybrid species can also allow direct identification of both ancestral parental lineages, if sufficient intraspecific sampling is undertaken, as in allopolyploids of *Gossypium* (Wendel, 1995). Evidence of such bidirectional ITS repeat-type fixation in *Gossypium* requires a note of caution for plant phylogeneticists, however. Misleading rDNA trees can result if lineages arise from populations of hybrid species that are fixed for nrDNA repeat-types of different parental origin. Species derived from such differentially fixed hybrid populations will not form a monophyletic group in the nrDNA trees but will group separately with the non-hybrid parent lineage that bears the orthologous nrDNA repeat-type. If, however, populations persist with individuals that maintain both parental ITS repeat-types, then inclusion of these sequences in the phylogenetic analysis (treated as separate OTUs) can resolve unambiguously the hybrid origin and biparentage of the hybrid lineage. It is important to note that with or without comprehensive sampling, reticulation events will always lead to evolutionary patterns that can be potentially misinterpreted in a dichotomous tree framework, regardless of the set of characters chosen for phylogenetic analysis.

Chimeric ITS repeat-types that combine distinctive motifs of ITS sequences from different lineages have been documented in species of *Gossypium* (Wendel, in press) and *Amelanchier* (Campbell, Wojciechowski, Baldwin & Donoghue, unpublished). These mosaic ITS sequences appear to have arisen by partial gene conversion or recombination between divergent paralogues brought together by hybridization. Formation of such "hybrid" sequences poses problems similar to those that can be expected in phylogenetic analysis of nuclear genes in general. Enhanced sampling of ITS repeat-types within individuals and across species and hybrids has improved resolution of the origin of chimeric ITS sequences in *Amelanchier* (Campbell, Wojciechowski, Baldwin & Donoghue, unpublished).

Conclusions

Available data from several angiosperm groups demonstrate that the ITS region can provide a valuable set of characters for addressing lower-

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FIGURE 8. Phylogenies of *Viburnum* (Adoxaceae) based on different data sets, reduced to a common set of taxa to illustrate areas of incongruence and implications for the evolution of fruit color (drawn using MacClade; Maddison & Maddison, 1992). —A. Morphological tree (based on Donoghue, 1983).—B. cpDNA restriction site tree (based on Donoghue & Sytsma, 1993, unpublished).—C. ITS sequence tree (based on Donoghue & Baldwin, 1993, unpublished). In each tree, capitalized terminal taxa are sections of *Viburnum* (except *Sambucus*). The remaining five taxa (all lower case) are representative species of the large section *Odontotinus* (OD), which appears not to be monophyletic. Open boxes/branches represent taxa or lineages with fruits that undergo a prolonged red phase before turning purple at maturity; lightly stippled boxes/branches symbolize taxa or lineages with fruits in which the red phase is greatly reduced or missing, and the shift to purple color occurs rapidly and synchronously; dark boxes/branches represent taxa or lineages with fruits that are red at maturity; hatched lines indicate that there are equally parsimonious state assignments (Maddison & Maddison, 1992). Note that cpDNA and ITS data both imply that *V. acerifolium* is not directly related to other New World species with purple fruits, as suggested by morphology. Instead, it appears to be more closely related to Asian species with red fruits.

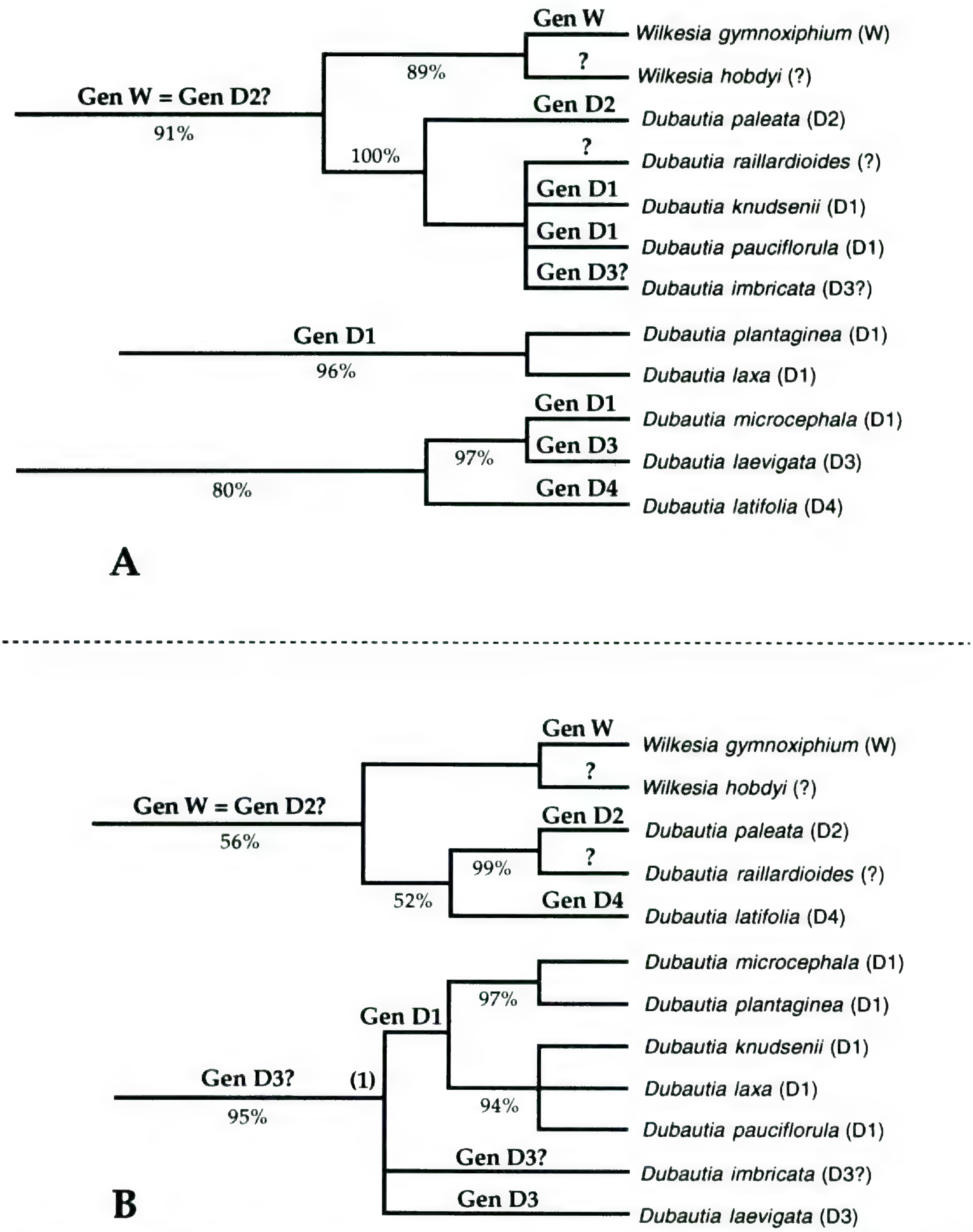

FIGURE 9. All Kaua'i lineages of the Hawaiian silversword alliance (Asteraceae: Madiinae) excised from phylogenies based on DNA evidence. Nuclear chromosomal arrangements (Carr & Kyhos, 1986, pers. comm.) are superimposed on the branches according to parsimony (if ambiguous, the earliest unambiguous possession of an arrangement is shown).—A. cpDNA restriction site tree (Baldwin, 1989; Baldwin et al., 1990).—B. ITS sequence tree (Baldwin & Robichaux, 1995; Baldwin, unpublished). Percentages below branches are bootstrap values. Genomic abbreviations (D1, D2, D3, D4, W) follow Carr & Kyhos (1986) with correction (Carr & Kyhos, pers. comm.). None of these chromosomal arrangements except D4 is known from other species of the silversword alliance. All species shown have the same chromosome number (n = 14; Carr, 1985; Carr & Kyhos, 1986). Question marks indicate an uncertain genomic arrangement. Available data are consistent with identity of genomes D2 and W, but confirmation from additional hybrids is needed (Carr & Kyhos, 1986, pers. comm.). Provisional assignment of D3 to *Dubautia imbricata* is based on high pollen stainability (99%) of a putative natural hybrid between this species and *D. laevigata* (D3) (Carr, 1985). If *D. imbricata* does possess the D3 chromosomal arrangement, then reconstruction of D3 as the

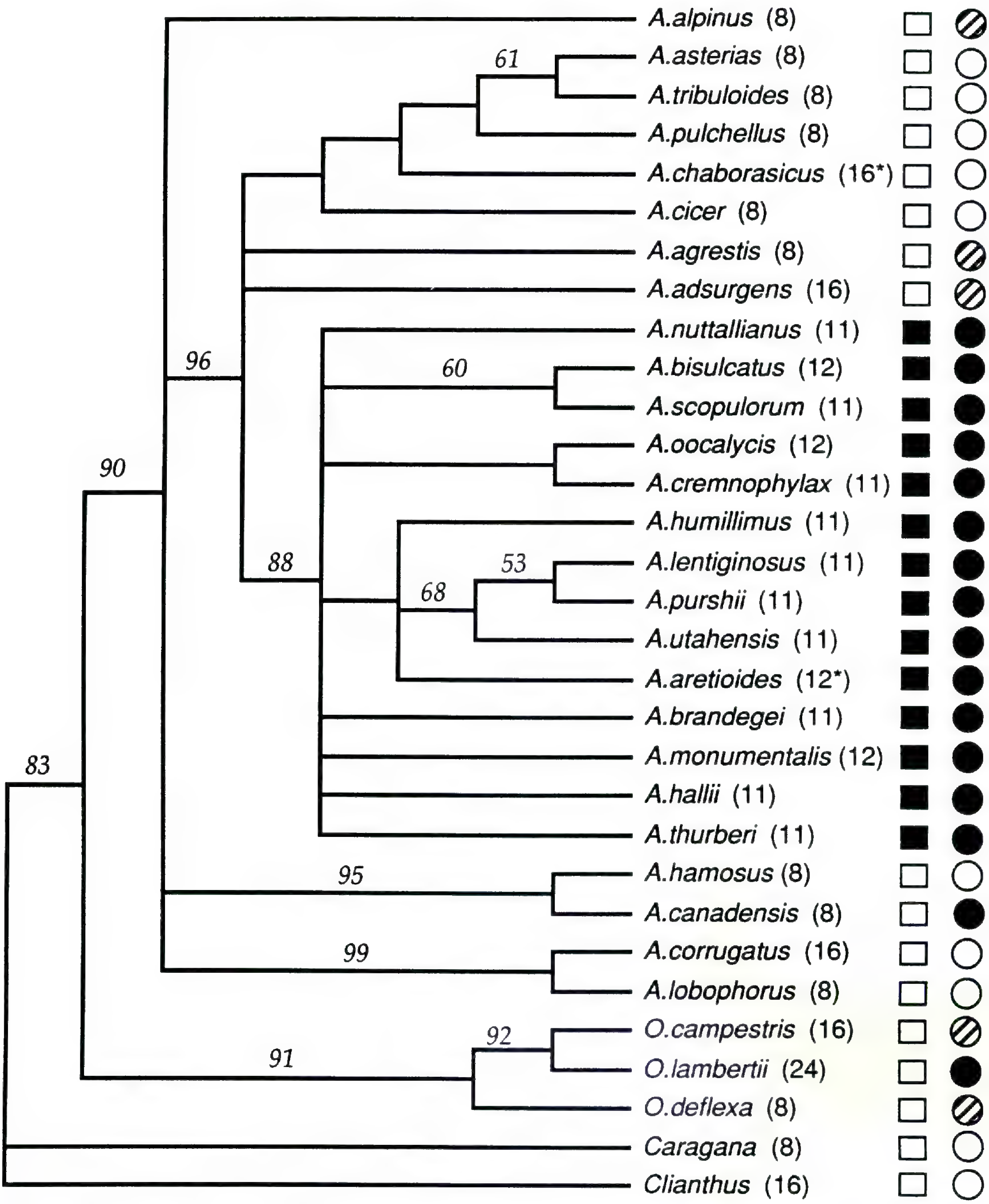

FIGURE 10. Strict consensus of the maximally parsimonious trees based on ITS sequences from 26 species of *Astragalus* and five outgroup taxa in *Caragana*, *Clianthus*, and *Oxytropis* (reprinted with permission from Wojciechowski et al., 1993). Bootstrap values are shown above the branches. Haploid chromosome numbers follow the taxon name. Asterisks indicate that the chromosome number of a species is unknown and that the number from other species in that section is given instead. Open squares indicate euploid species; black squares represent aneuploid species. Open circles indicate Old World taxa; hatched circles represent circumboreal species; black circles designate New World species. Note well-supported monophyly of the New World aneuploids, a morphologically cryptic clade that is corroborated by cpDNA results (Sanderson & Doyle, 1993).

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ancestral condition in the lower ITS lineage would be unambiguous. D3 differs from D1 and D5 by different single reciprocal translocations and bridges the two latter arrangements. D5 is the basal condition in the sister group (not shown) to the lower of the two ITS lineages. Note that the ITS relationships are consistent with single origins of D1 and D3, unlike the cpDNA resolution.

level phylogenetic questions in flowering plants. The lack of identified nuclear DNA regions that possess such favorable properties for phylogenetic studies of closely related plant species makes the ITS region a particularly important new resource for angiosperm systematists. ITS data should play an especially useful role in angiosperm studies by offering independent assessment of lower-level phylogenetic hypotheses based on morphology or cpDNA evidence.

One of the most attractive aspects of using the ITS region for plant molecular phylogenetic studies is the ease with which sequences of both spacers can be obtained and interpreted. The small size, highly conserved flanks, high copy number, rapid concerted evolution, and length conservation of angiosperm ITS sequences greatly aid their PCR amplification, sequencing, alignment, and phylogenetic analysis (see Baldwin, 1992). A minor sequencing effort can yield complete sequences of ITS-1 and ITS-2 from many taxa. In turn, these sequences may provide sufficient potentially informative variation for robust resolution of phylogenetic relationships, as now shown in several groups of closely related angiosperms (see above). Predominance of site-substitutions over indel mutations and interspersion of conserved and variable sites promote ITS sequence alignment and use of ITS indels as characters in phylogenetic analysis. Rapid homogenization of nrDNA repeats by concerted evolution can allow reconstruction of species relationships from direct sequences of the ITS region, whereas limited persistence of divergent ITS paralogues can provide direct evidence of hybridization, independent estimates of phylogeny, or a novel criterion for tree rooting.

The primary limitation of ITS data for phylogenetic studies in angiosperms is the small number of characters from these short spacers. Four-taxon simulations by Huelsenbeck & Hillis (1993) suggest that sequences of such short length are, under most conditions and types of analysis, less effective for accurate tree reconstruction than longer sequences. Useful variation must be more highly concentrated within a set of ITS-1 and ITS-2 sequences than in longer DNA regions in order to achieve the same level of phylogenetic resolution and support. Further constraints on the number of useful ITS characters can be imposed by the need to delete small indel regions from phylogenetic analysis because of uncertain sequence alignment. These limitations may require that data from other sources (e.g., morphology) be combined with ITS evidence to obtain enough characters for well-supported phylogenetic resolution (see Kluge, 1989; Barrett et al., 1991; Donoghue & Sanderson, 1992).

Where incongruence statistics have been applied (Campbell et al., in press; Kim & Jansen, 1994), ITS data have appeared substantially congruent with other independent data sets. Some relationships suggested by ITS trees have been seen as questionable based on apparent conflict with multiple lines of congruent evidence (e.g., Kim & Jansen, 1994; Soltis & Kuzoff, in press). In most of these cases, mere homoplasy in the ITS data sets may be responsible for the conflicts. As discussed above, differential fixation or partial conversion of non-homologous repeat-types in hybrids, or in a set of species derived from a polymorphic ancestor, could lead to a well-supported ITS phylogeny that does not accurately reflect species relationships, as in *Gossypium* (Wendel, 1995; in press). Hybridization or lineage sorting can, however, lead to deviation between *any* tree derived from a single gene or organellar genome and a species tree and are therefore not a special liability of ITS data. A confident assessment of organismal phylogeny can come only from examination of multiple lines of evidence, both in parallel and in combination.

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AN EVALUATION OF THE USEFULNESS OF THE SMALL HEAT SHOCK GENES FOR PHYLOGENETIC ANALYSIS IN PLANTS[1]

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ABSTRACT

Sequences from chloroplast DNA and rDNA genes have proved useful in estimating phylogenetic relationships among plants. However, the coding regions of rDNA evolve slowly and are not useful at lower levels of analysis. There are chloroplast-encoded genes that evolve at a higher rate, but since chloroplast DNA is uniparentally inherited, nuclear markers are also needed when introgression or hybridization have occurred. There is then a need for additional nuclear genes that can be used in phylogenetic analysis. Nuclear genes have been largely unused in phylogenetic analysis for several reasons, including the difficulty of distinguishing orthologous from paralogous genes and the problems that can ensue from constructing phylogenetic trees from data matrices that contain both types of genes. This paper is an evaluation of the potential of the genes encoding the small heat shock proteins for phylogenetic analysis of plants. The small heat shock genes in plants are a super gene family composed of four gene families. In this paper I present restriction site analysis of the small heat shock genes from the Brassicaceae, and similarity, rate of evolution, and phylogenetic analysis of small heat shock gene sequences from monocots and dicots. I show that these genes possess the necessary genetic variation for phylogenetic analysis and that the gene families are easily distinguishable from each other, and that, in at least some of the gene families, orthologous and paralogous genes do not pose a problem for phylogenetic analysis.

In recent years the availability of molecular data has vastly increased our ability to test hypotheses of both phylogenetic relationships among plant groups and population-level evolutionary processes. These studies, with few exceptions, have relied primarily on chloroplast DNA (cpDNA) and nuclear ribosomal DNA (rDNA) (Clegg & Zurawski, 1992; Hamby & Zimmer, 1992). The large numbers of cpDNA sequences (Chase et al., 1993) across many taxonomic groups have enabled scientists to test hypotheses concerning rates of evolution of genes used as markers in phylogenetic analysis, in addition to addressing phylogenetic questions (Bousquet et al., 1992; Gaut et al., 1992). However, the recognition of the distinction between gene trees and species trees and the effects that hybridization can have on phylogenies constructed from uniparentally inherited genes (Dorado et al., 1992; Doyle, 1992; Pamilo & Nei, 1988; Rieseberg et al., 1990; Rieseberg & Brunsfeld, 1992; Rieseberg & Soltis, 1991) has led to the awareness that additional molecular markers are needed. In addition, there is concern over the effects of multiple changes at one site (Smith, 1989) and secondary structure and compensatory mutations (Dixon & Hillis, 1993) on phylogenetic reconstruction using rDNA sequences.

Systematists have been wary of using nuclear genes for phylogenetic analysis because many nuclear genes are members of multi-gene families (Doyle, 1993). The inability to distinguish orthologous and paralogous genes within a multi-gene family could undermine attempts to construct organismal relationships. Gene conversion and recombination within gene families will obscure organismal relationships, and this may not be immediately obvious from the cladograms (Sanderson & Doyle, 1992). Thus, prior to its use in phylogenetic analysis the evolutionary dynamics of a gene should be examined. In this paper the small

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heat shock protein genes are examined for their usefulness in phylogenetic reconstruction.

Organisms respond to heat stress with the rapid induction of the heat shock genes and subsequent production of heat shock proteins (hsp) that are necessary for thermal tolerance. The heat shock genes are members of multi-gene families based on the size of the proteins the genes encode. The small heat shock proteins are less than 30 kDa (kilo-Dalton) in size. The large heat shock proteins range from 60 to 100 kDa. The 90, 70, and 60 kDa proteins are the most extensively studied of all of the heat shock proteins. The large heat shock proteins are chaperone proteins, i.e., they help in the proper folding and translocation of other proteins (Becker & Craig, 1994; Gething & Sambrook, 1992). The function of the small heat shock proteins, however, remains elusive. In most organisms, other than plants, the majority of the heat shock proteins produced during stress are large. The small heat shock proteins are present, but are a small percentage of the total protein. In *Drosophila* there are just four small heat shock genes, and in *Saccharomyces* there is only one (Lindquist & Craig, 1988; Susek & Lindquist, 1989). In contrast, plants produce a variety of small heat shock proteins (Vierling, 1991). There are four small heat shock protein gene families in plants. The four classes are based on overall sequence similarity and cellular localization. There are two classes of proteins that localize to the cytosol, Class I and Class II. The third class of proteins is found in the endoplasmic recticulum, and the fourth in the chloroplast. The carboxyl-terminal domain is more conserved than the amino-terminal domain both within and across protein classes (Vierling, 1991). Vierling (1991) suggested that the four classes of small heat shock genes in plants may have evolved through gene duplications.

Materials and Methods

Cloning and Mapping of a Small Heat Shock Gene in *Brassica nigra*

Brassica nigra (CrGC accession number 2-1) seeds were obtained from the Crucifer Genetic Cooperative (CrGC, Madison, Wisconsin). Seeds were germinated and planted in Terra-Lite (Grace Horticultural Products, Cambridge, Massachusetts) and grown in growth chambers in the Washington University Biology Department Plant Growth Facility (St. Louis, Missouri). Plants were harvested when they were two to three weeks old. Leaf tissue was ground to a powder in liquid nitrogen and stored at −80°C. Total genomic DNA was isolated from individuals using a modified CTAB procedure with a phenol-chloroform extraction (King & Schaal, 1990).

Genomic DNA from *Brassica nigra* was cut with *Xba* I and was ligated into Lambda Zap vector (Stratagene, San Diego, California) also cut with *Xba* I. The lambda library was amplified in NM522 cells obtained from Stratagene. Lambda plaques were transferred from agar plates to nylon filters by the filter-lift procedure (Sambrook et al., 1989). Plaques of the heat shock gene were identified in the library using a cDNA clone of a small heat shock gene from *Glycine max* pCE53 (Schoffl & Key, 1982). The nylon (Magna nylon, MSI) filters were pre-hybridized and hybridized with hexamer-labeled pCE53 using standard protocols (King & Schaal, 1990) at moderate stringency: 2 × 15 min. with 2 × SSC 0.1% SDS at 25°C and 2 × 25 min. with 1 × SSC 0.1% SDS at 37°C (Sambrook et al., 1989). Plaques were isolated and pBluescript plasmids were obtained from the plaques according to the Stratagene in-vivo excision protocol.

Plasmid DNA was obtained by alkaline lysis followed by phenol extraction (Sambrook et al., 1989) and digested with the following restriction enzymes: *Acc* I, *Hind* III, *Sal* I, *Pst* I, *Eco* RI, *Xba* I, and *Xho* I (New England Biolabs). Reactions were conducted according to manufacturer's recommendations. Restriction digests were electrophoresed in 0.85% agarose gels in tris-acetate buffer. The DNA was visualized under UV light by staining with ethidium bromide. The DNA was transferred to nylon filters using standard Southern blotting procedures. The coding region of the genes was identified by hybridization of the plasmid DNA digests with radio-labeled cDNA clone of a 17.6 kDa hsp from *Arabidopsis thaliana* (Helm & Vierling, 1989).

Survey of Genetic Variability

Genomic DNA was isolated, using the CTAB extraction procedure described above, from each of 15 individuals of *Brassica nigra* (CrGC #2-1). Genomic DNAs were digested with *Eco* RI, and the restriction digests were electrophoresed in 0.8% agarose gels in tris-acetate buffer and transferred to nylon membrane using standard procedures. Nylon filters were hybridized with the hexamer-labeled small heat shock gene clone from *B. nigra*, described above. Filters were washed 2 × 15 min. with 2 × SSC 0.1% SDS at 65°C and 2 × 45 min. with 0.1 × SSC 0.1% SDS at 65°C and

exposed to x-ray film with intensifying screens at −80°C for three to five days.

To assess within-population restriction site variation in the small heat shock genes, seeds of *Brassica oleracea*, *Cardamine keyserri*, and *Rorippa schlechteri* were collected in Papua New Guinea. Collections of seeds (at least 20 seeds from each of 10 individuals per population) were made in and around the village of Safalitikin, Urapmin, Sandaun Province. Voucher specimens were deposited in the herbarium at UPNG and at MO.

Seeds were germinated and grown as described above. When plants were approximately four weeks old, leaves were harvested and stored at −80°C. Eight individuals from two populations of *Rorippa schlechteri* and eight individuals from one population each of *Brassica oleracea* and *Cardamine keyserri* were surveyed for within-population variation. DNA from individual plants was digested with *Ban* I, *Eco* RI, *Hpa* I, *Hinf* I, *Hind* III, *Rsa* I, *Pst* I, and *Taq* I (New England Biolabs) according to manufacturer's instructions. Restriction digests were electrophoresed in 0.8–1.2% agarose gels in tris-acetate buffer. DNA was transferred to nylon membranes and hybridized with the hexamer-labeled small heat shock clone from *B. nigra*, described above. Filters were washed 2 × 15 min. with 2 × SSC 0.1% SDS at 65°C and 2 × 45 min. with 0.1 × SSC 0.1% SDS at 65°C and exposed to x-ray film with intensifying screens at −80°C for three to five days.

SURVEY OF THE *BRASSICA* TRIANGLE AND OTHER BRASSICACEAE

Restriction sites in the small heat shock genes were surveyed among the following species: *Rorippa schlechteri*, *Raphanus sativus* (CrGC #7-1), *Cardamine keyserri*, *Arabidopsis thaliana* (CrGC #9-3), *Brassica nigra* (CrGC #2-1), *B. oleracea* (CrGC #3-1), *B. rapa* (CrGC #1-1), *B. carinata* (CrGC #6-1), *B. juncea* (CrGC #4-1), and *B. napus* (CrGC #5-1). The *Brassica* triangle is composed of the diploids *B. nigra*, *B. oleraceae*, and *B. rapa* and the amphidiploids *B. carinata*, *B. juncea*, and *B. napus* (Pakrash & Hinata, 1980).

Genomic DNA from at least two individuals from each species was isolated and cut with *Bam* HI, *Eco* RI, *Fok* I, *Hind* III, *Hae* III, *Pst* I, *Rsa* I, *Sal* I, and *Xba* I restriction enzymes. Digested DNAs were separated in 0.8% agarose gels, transferred to nylon membranes, and hybridized with the hexamer-labeled small heat shock clone from *Brassica nigra*, described above. Filters were washed 2 × 15 min. with 2 × SSC 0.1% SDS at 65°C and 2 × 45 min. with 0.1 × SSC 0.1% SDS at 65°C and exposed to x-ray film with intensifying screens at −80°C for three to five days.

A portion of the gene encoding the small heat shock protein that is localized to the chloroplast was amplified from the genomic DNA of *Arabidopsis thaliana*, *Cardamine keyserri*, *Brassica nigra*, *B. oleracea*, *B. napus*, and *B. juncea* using Taq DNA polymerase, the manufacturer's buffer (Boehringer Mannheim), and primers internal to the coding sequence of the *A. thaliana* gene (Osteryoung et al., 1993). The primers were from base pairs 151–169 and 765–782 (using the start of the coding sequence as base pair 1). The DNA was amplified for 35 cycles with the following conditions: 94°C 1 min., 50°C 2 min., and 72°C 2 min. Products were visualized after electrophoresis in a 0.9% agarose gel with ethidium bromide.

SEQUENCE DATA ANALYSIS

Gene sequences were obtained from GenBank (see Table 1). Sequences were compared using the Gap program in the Genetic Computer Group (GCG) computer package. Alignments were generated using Pileup in GCG and were refined by hand using Lineup (see Appendix). The DNA sequences coding for the transit peptides were not used in these analyses. Synonymous and nonsynonymous substitutions were calculated using the computer program of Li (1993). Phylogenetic analyses of the small heat shock gene sequences were done using PAUP (3.1.1) (Swofford, 1993). Heuristic searches were conducted using 100 random addition taxon replicates with MULPARS and TBR branch swapping. A bootstrap analysis with 100 replicates was performed to assess support for branches.

RESULTS

A full-length genomic clone encoding a cytosolic small heat shock protein was isolated. A restriction map of that clone is presented in Figure 1. The restriction enzyme *Eco* RI cleaves this clone twice. An *Eco* RI digest of genomic DNA of *Brassica nigra* probed with the clone of the small heat shock gene is presented in Figure 2. The sizes of the genomic *Eco* RI fragments in Figure 2 correspond to those predicted from the plasmid restriction map in Figure 1. The results of this Southern blot and others (data not shown) indicate that this gene is present in a single copy in *B. nigra*.

POPULATION-LEVEL VARIATION

To determine if there is variability in the small heat shock gene within populations, I conducted a survey in *Brassica nigra*, *B. oleracea*, *Cardamine*

Genomic heat shock clone from *Brassica nigra*

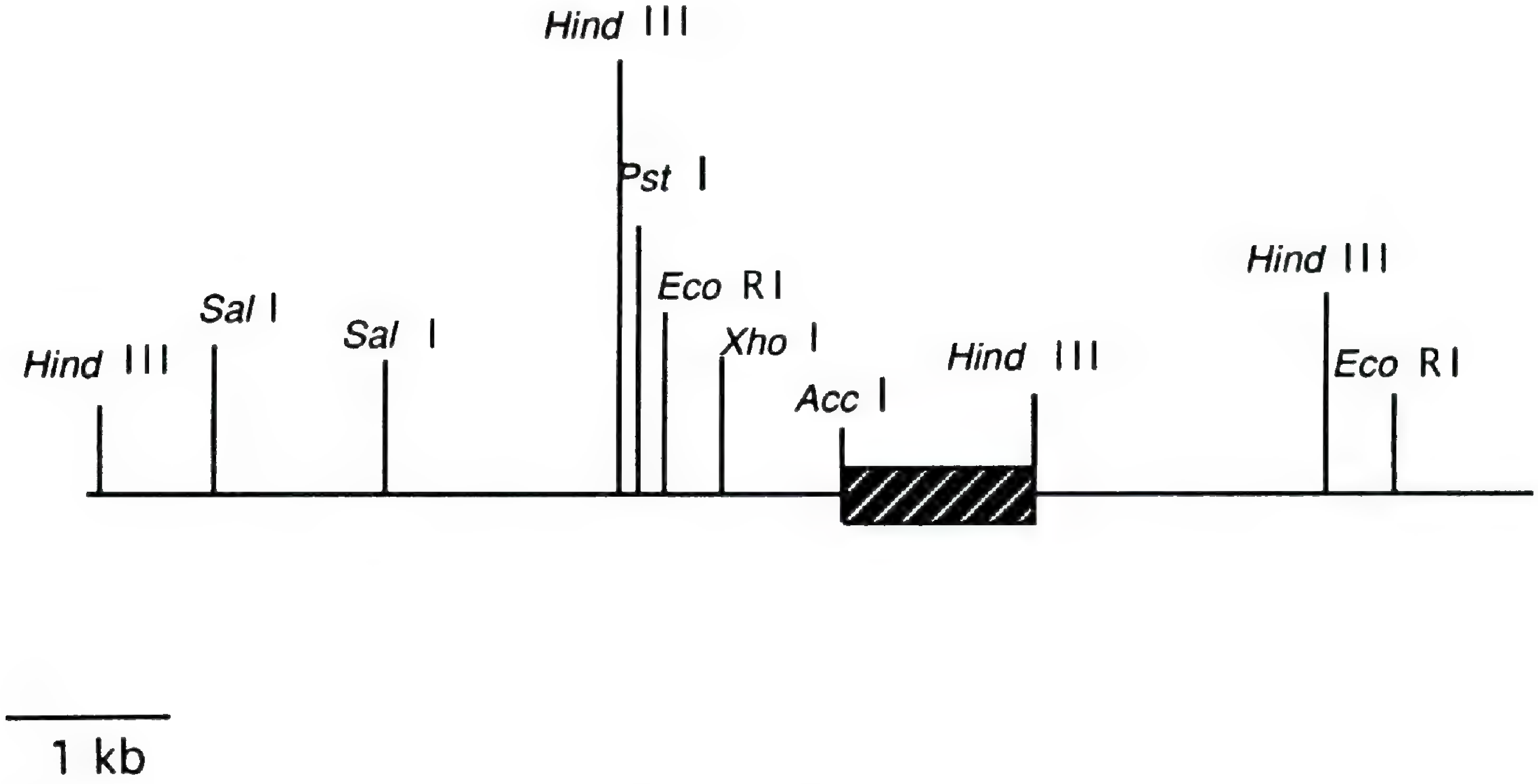

FIGURE 1. Restriction map of a *Brassica nigra* small heat shock gene. The shaded box represents the coding region.

TABLE 1. Sources of sequences used in phylogenetic analyses of small heat shock genes.

Species	Protein	GenBank accession	Reference
A. Genes for the chloroplast-localized proteins:			
Triticum aestivum Percival	Hsp 26A	X58280	Weng et al., 1991
Triticum aestivum	Hsp 26B	X67328	unpublished
Pisum sativum Poir.	Hsp 21	X07187	Vierling et al., 1988
Glycine max Merr.	Hsp 22	X07188	Vierling et al., 1988
Petunia hybrida Vilm.	Hsp 21	X54103	Chen & Vierling, 1991
Arabidopsis thaliana B. Heyne	Hsp 21	X54102	Chen & Vierling, 1991
B. Genes for endomembrane-localized proteins:			
Pisum sativum	Hsp 22	M33898	Helm et al., 1993
Glycine max	Hsp 22	X63198	Helm et al., 1993
C. Genes for the class I cytosolically-localized proteins:			
Zea mays Doebley & Iltis	Hsp 17.2	X65725	unpublished
Pisum sativum	Hsp 18.1	M33899	Lauzon et al., 1990
Arabidopsis thaliana	Hsp 17.6	X16076	Goping et al., 1991
D. Genes for the class II cytosolically-localized proteins:			
Zea mays	Hsp 17.5	X54076	Goping et al., 1991
Zea mays	Hsp 17.8	X54075	Goping et al., 1991
Triticum aestivum	Hsp 17.3	X58279	Weng et al., 1991
Pisum sativum	Hsp 17.7	M33901	Lauzon et al., 1990
Glycine max	Hsp 17.9	X07159	Raschke et al., 1988
Arabidopsis thaliana	Hsp 17.6	X63443	Barling et al., 1992
Pharbatis nil Roth	Hsp 18.8	M99430	Krishna et al., 1992
Pharbatis nil	Hsp 17.2	M99429	Krishna et al., 1992

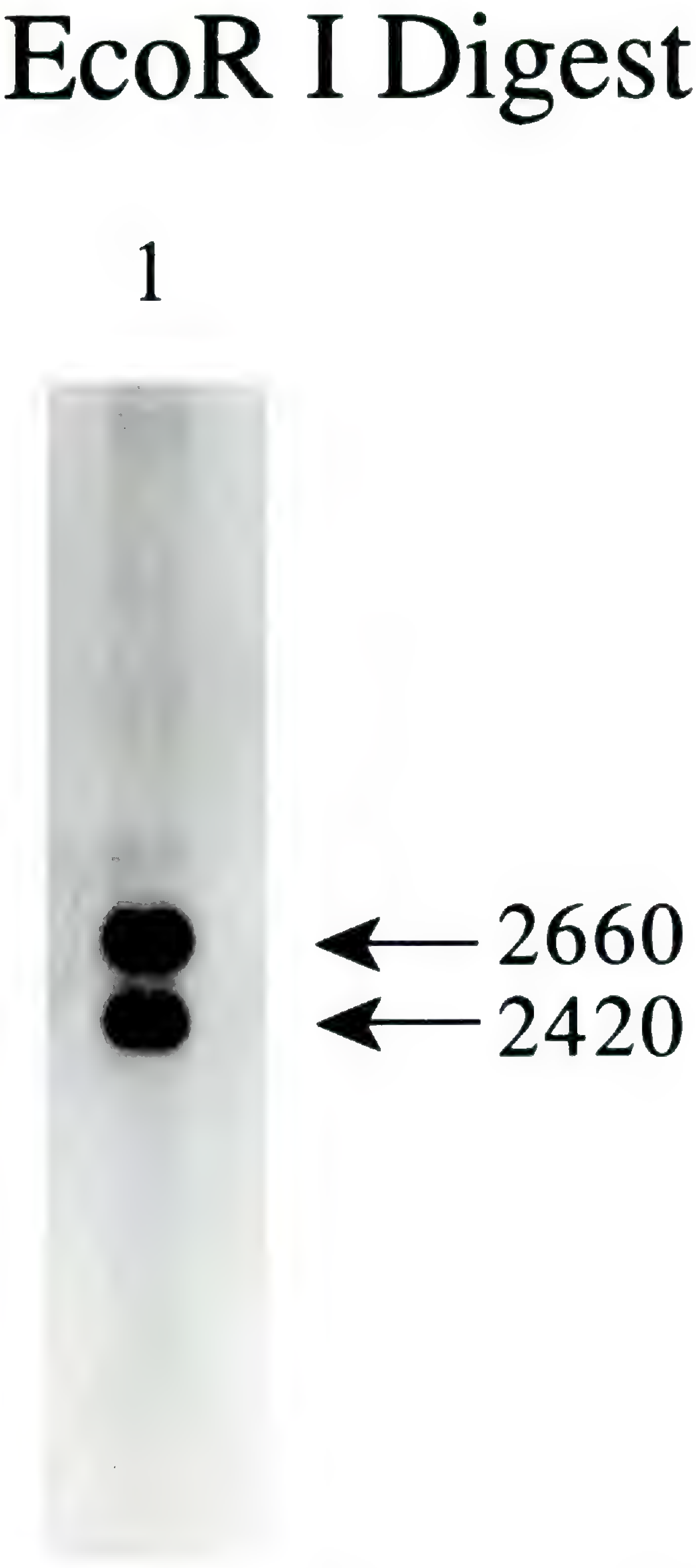

FIGURE 2. *Eco* RI digest of *Brassica nigra* genomic DNA probed with the clone of the *B. nigra* small heat shock gene.

keyserri, and *Rorippa schlechteri*. DNA from 15 individuals of the CrGC rapidly cycling population of *B. nigra* was digested with *Eco* RI. This blot was probed with the full-length heat shock clone (containing both the coding and the flanking regions), and no polymorphisms were detected (data not shown). Populations of *B. oleracea*, *R. schlechteri*, and *C. keyserri* were also surveyed. DNAs from eight individuals from each of these populations were digested with eight restriction enzymes, and the blots were probed with the full-length probe. Again, there was no detectable variation for the small heat shock gene in these populations (data not shown).

VARIATION AMONG MEMBERS OF THE BRASSICACEAE

Restriction site variation in the small heat shock gene was assessed among the members of the *Brassica* triangle and among other genera of the Brassicaceae, *Arabidopsis thaliana*, *Cardamine keyserri*, and *Rorippa schlechteri*. The relationships between the members of the *Brassica* triangle were established with cytological, morphological, and genetic evidence (Pakrash & Hinata, 1980), and analysis of the chloroplast genomes established the maternal and paternal parents of the amphidiploids (Erickson et al., 1983; Palmer et al., 1983). *Brassica carinata* has the chloroplast genome of *B. nigra*, and *B. juncea* has the chloroplast genome of *B. rapa*. Although the data for *B. napus* are equivocal, it appears that in at least some accessions *B. rapa* is the maternal parent. DNAs from each of these species were digested with five restriction enzymes and probed with the full-length clone and with a clone containing only the coding region. No restriction site polymorphisms were found within the coding regions among members of the *Brassica* triangle studied, including *Raphanus* (data not shown). However, variation in restriction sites was detected within the coding region among genera in the Brassicaceae. When the Southern blots were hybridized with the full-length clone, restriction site variation was observed among the members of the *Brassica* triangle, indicating sequence divergence in the flanking region (Fig. 3).

Primers internal to the coding region were used to amplify the gene encoding the small heat shock protein localized to the chloroplast from six species of the Brassicaceae. A single band was present in *Brassica nigra*, *B. oleraceae*, *Cardamine keyserri*, *Arabidopsis thaliana*, *B. juncea*, and *B. napus* (Fig. 4). This indicates that this is a single-copy gene and that PCR (Polymerase Chain Reaction) amplification is easily accomplished.

SEQUENCE ANALYSIS

Sequences from different gene families were compared within *Arabidopsis thaliana* and within *Pisum sativum* (Table 2). In this analysis, the gene for the chloroplast-localized protein in *A. thaliana* was compared to other genes from *A. thaliana* and the gene for the chloroplast protein in *P. sativum* was compared to other genes in *P. sati-*

vum; no interspecific comparisons were made. The gene sequences for the endomembrane proteins and both classes of the cytosolically localized protein are less than 50% similar to the gene for the protein localized to the chloroplast. In contrast, the genes for the chloroplast proteins are more than 50% similar to each other based on interspecific comparisons (Table 3), even between monocots and dicots. Genes from each class or family are more similar to each other than they are to other small heat shock proteins in the same species. It is clear from these comparisons that there is no, or very little, gene conversion across gene families.

Analysis of synonymous (Ks) and nonsynonymous (Ka) substitutions (Table 4) indicates that the small heat shock genes are evolving at rates comparable to that reported for other nuclear genes (Wolfe et al., 1989). In the comparison among the grasses, Ks is not saturated, and Ka is, as expected, much lower than Ks. In the comparisons among the dicots (Table 4), Ks is saturated, reflecting the more distant relationships of these species. The Ka calculated for the small heat shock genes is comparable to the rates reported for the gene for chalcone synthase (Wolfe et al., 1989). Rates of synonymous and nonsynonymous substitutions were determined for three of the gene classes for *Arabidopsis thaliana* and *Glycine max*. The complete sequence of the gene for the chloroplast-localized protein in *G. max* has not been isolated, precluding a comparison of the rate for the entire gene. The gene classes are all evolving at approximately the same rate (Table 4).

In a phylogenetic analysis of the aligned gene sequences of the four major classes of proteins (the Class I and Class II cytosolically localized proteins, the endomembrane proteins, and the chloroplast proteins), the sequences of each gene family form well-supported clades (Fig. 5). Third positions of codons were omitted from the analysis because the rate analysis indicated that substitutions at these positions are saturated. A single tree of 936 steps with a consistency index (CI) of 0.640 was found in all the 100 heuristic searches. The order of gene duplications is not known, so the tree is arbitrarily rooted with the Class I sequences. A tree with the same topology was obtained using aligned amino acid sequences for the same genes (data not shown). Within each gene family, the gene phylogenies are roughly congruent with the species phylogenies. However, the taxon sampling is not the same in each gene family. There is weak support (i.e., bootstrap values less than 50%) for some of the branches within the Class II gene family.

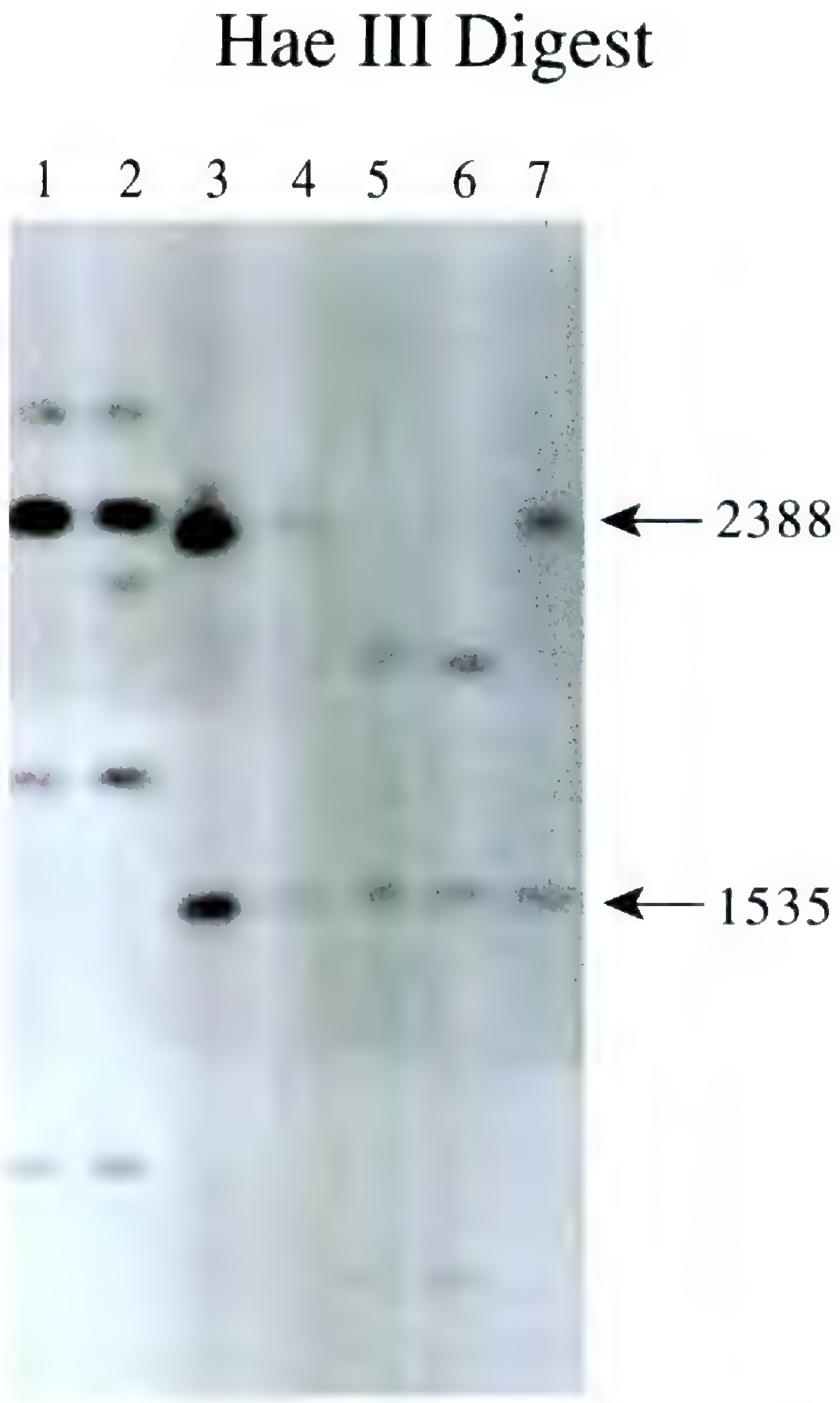

FIGURE 3. *Hae* III digest probed with the *B. nigra* small heat shock gene. Lane 1, *B. nigra*; lane 2, *B. juncea*; lanes 3 and 4, *R. sativus*; lanes 5 and 6, *A. thaliana*; lane 7, *B. oleracea*.

DISCUSSION

Phylogenetic analysis of molecular data sets has enabled evolutionary biologists to approach many questions not answerable with morphological data alone. In plant evolutionary studies, rDNA and cpDNA are the most frequently used molecular markers. Chloroplast DNA is useful in reconstructing organismal relationships above the species level,

TABLE 2. Similarity of the gene coding for the chloroplast-localized protein to other small heat shock proteins in *Arabidopsis thaliana* and *Pisum sativum*.

	A. thaliana	*P. sativum*
Class I	47.9%	44.5%
Class II	45.0%	47.6%
Endomembrane-localized		41.6%

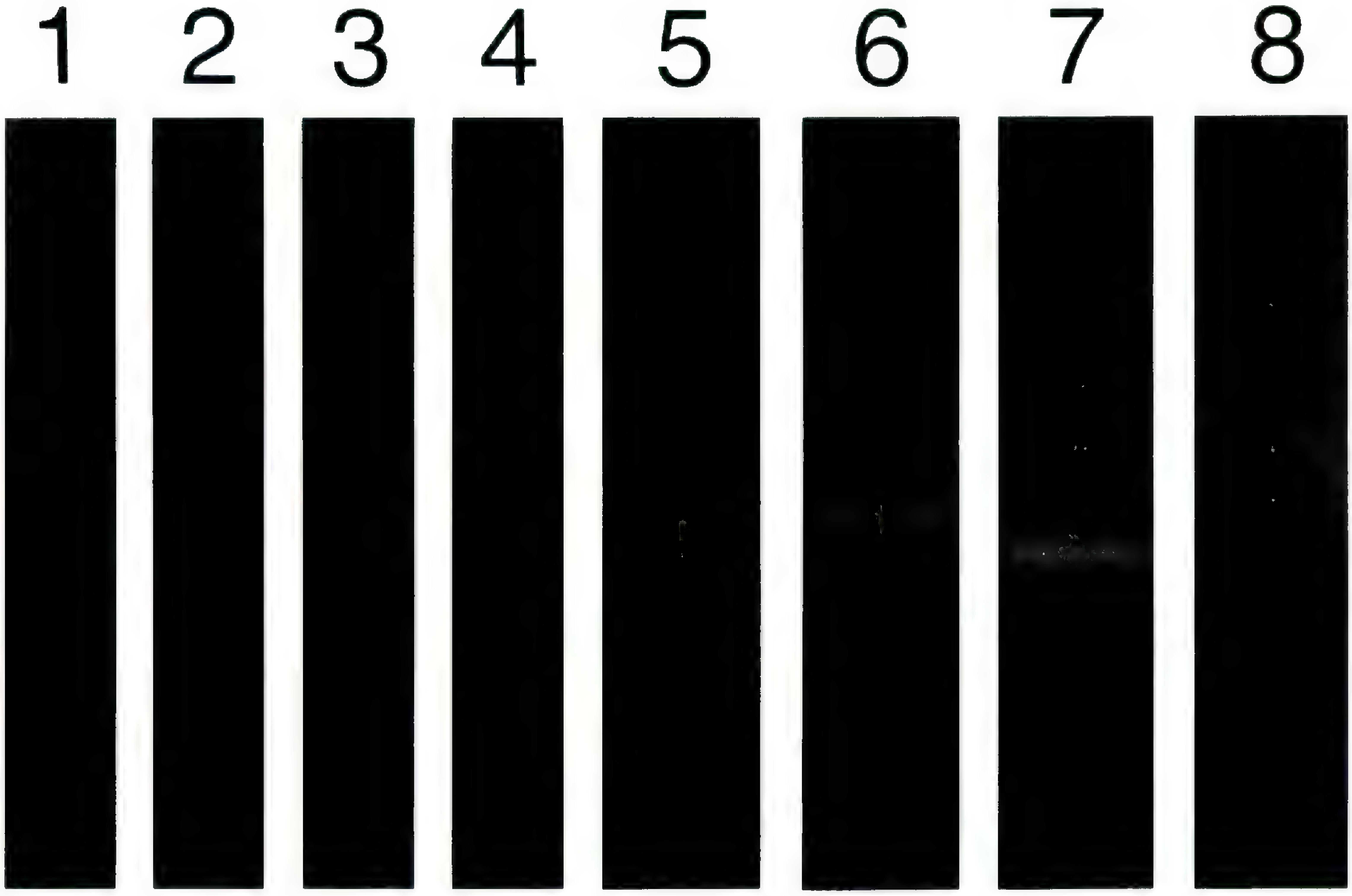

FIGURE 4. Agarose gel stained with ethidium bromide. PCR products amplified using primers for the gene encoding the chloroplast protein. Lane 1, *Brassica nigra*; 2, *B. napus*; 3, *R. schlechteri*; 4, *B. carinata*; 5, *B. oleracea*; 6, *B. juncea*; 7, *A. thaliana*; 8, *C. keyserri*. The *A. thaliana* PCR product is 590 bp.

through both restriction site and sequence analysis, due to its low rate of evolution. However, because chloroplasts are typically uniparentally inherited, the usefulness of cpDNA data sets may be limited when there has been hybridization and introgression. For example, some individuals of *Helianthus annuus* have the rDNA of one species and the cpDNA of another (Dorado et al., 1992; Rieseberg et al., 1990; Rieseberg & Brunsfeld, 1992). Phylogenetic relationships inferred from a tree based on cpDNA can contradict those inferred from an rDNA tree. Patterns of cpDNA distribution can also reflect geographical relationships rather than species designations (Brunsfeld et al., 1992; Matos, 1992; Soltis et al., 1992). Studies such as these make clear the need for additional nuclear markers for use in phylogenetic studies.

Many researchers have avoided using nuclear markers because of the concern that many nuclear genes are members of multi-gene families and that gene conversion among these genes would obscure orthologous (due to speciation and reflecting organismal phylogeny) and paralogous (due to gene duplication and reflecting gene phylogeny) relationships. In a recent review, Doyle (1993) stated that "The nuclear genome would appear to have limitless potential for phylogenetic studies . . . yet it remains largely untapped as a source of DNA characters." Gene duplication and conversion are widely believed to be important in the evolution of multi-gene families (Ohta, 1987, 1988). Frequent gene conversion among members of multi-gene families maintains sequence similarity but also restricts the independent evolution of individual genes and results in concerted evolution. Two forces will maintain high levels of similarity among gene sequences: selection and gene conversion. In gene conversion, related gene sequences are homogenized within a genome. Using computer simulation, Sanderson & Doyle (1992) examined the difficulty in determining true species relationships using sequence data from multi-gene families when there has been gene conversion and recombination. The results of this study suggest that the true species tree can be inferred when gene conversion is high (i.e., as in rDNA) and when gene conversion is low, but that intermediate levels of gene conversion will make it difficult to distinguish orthologous and paralogous genes and will obscure species relationships. An analysis of sequences of the nuclear gene

TABLE 3. Similarity of the nucleotide sequences of the genes for the chloroplast-localized proteins.

	P. sativum	*G. max*	*P. hybrida*	*A. thaliana*	*T. aestivum A*
P. sativum					
G. max	82.4%				
P. hybrida	65.7%	72.3%			
A. thaliana	63.1%	69.2%	63.3%		
T. aestivum A	52.2%	59.6%	54.7%	56.2%	
T. aestivum B	52.6%	59.2%	54.1%	55.5%	96.5%

family encoding the small subunit of ribulose 1,5-bisphosphatase carboxylase (*rbc*S) in 17 genera, including green algae and cyanobacteria, demonstrated gene conversion among the *rbc*S genes (Meagher et al., 1989). However, Meagher's analysis also indicates that in spite of gene conversion, *rbc*S sequences can be used in some instances to infer generic and higher-level relationships.

There is no evidence for widespread gene conversion across gene families of the small heat shock genes, although they appear to be related to each other through gene duplications. The small heat shock genes have a higher level of similarity within families across species than they do within species across families; this most likely reflects selective constraints. Similar results were reported in an analysis of the amino acid sequences of the small heat shock proteins (Vierling, 1991). Regions of high similarity within a genome are necessary for gene conversion to occur. Gene conversion would result in higher levels of similarity among genes across families, within a species. The small heat shock genes are evolving at a rate comparable to other nuclear genes and faster than genes encoded in the chloroplast (Wolfe et al., 1989). A comparison of the nuclear *Adh* genes from *Zea mays*, *Triticum aestivum*, and *Hordeum vulgare* found a Ks of 0.66 and a Ka of 0.03 (Wolfe et al., 1989). Between the Solanaceae and the Brassicaceae, the Ks was greater than 2.50, and Ka was 0.10 (Wolfe et al., 1989).

In a tree of representatives of all the small heat shock gene families, each gene family formed a well-supported clade (bootstrap values of 99–100%). However, there is a lack of support for some of the branches within the Class II cytosolic gene clade. In *Pharbatis nil* the 17.2 kDa protein is induced by changes in photoperiod and heat shock, and the 18.8 kDa protein is induced only by heat shock. Due to the lack of sequence data for the small heat shock genes in related species, it is unclear whether these two genes are the products of a recent or more ancient duplication. Hence, it is not possible to determine the paralogous and orthologous relationships between these genes and the other Class II sequences. It is this type of uncertainty that can make inferring organismal relationships problematical and perhaps unreliable.

The chloroplast-localized small heat shock genes may prove the most useful in phylogenetic analysis. These genes are present in a single copy in the Brassicaceae, and the gene tree reflects species relationships. In addition, these genes are longer than the other small heat shock genes and thus provide more characters. The sequences for the transit peptides were not used in these analyses due to the difficulty of aligning the sequences across distantly related taxa. However, the rapid evolution

TABLE 4. Rates of synonymous (Ks) and nonsynonymous (Ka) substitutions among the small heat shock genes.

	Ks	Ka
Z. mays vs. *T. aestivum*		
Chloroplast-localized	0.44 (± 0.13)	0.09 (± 0.02)
Class II Gene	0.36 (± 0.09)	0.08 (± 0.02)
A. thaliana vs. *P. hybrida*		
Chloroplast-localized	>2	0.16 (± 0.02)
A. thaliana vs. *G. max*		
Class I cytosolic	>2	0.21 (± 0.02)
Class II cytosolic	>2	0.22 (± 0.02)

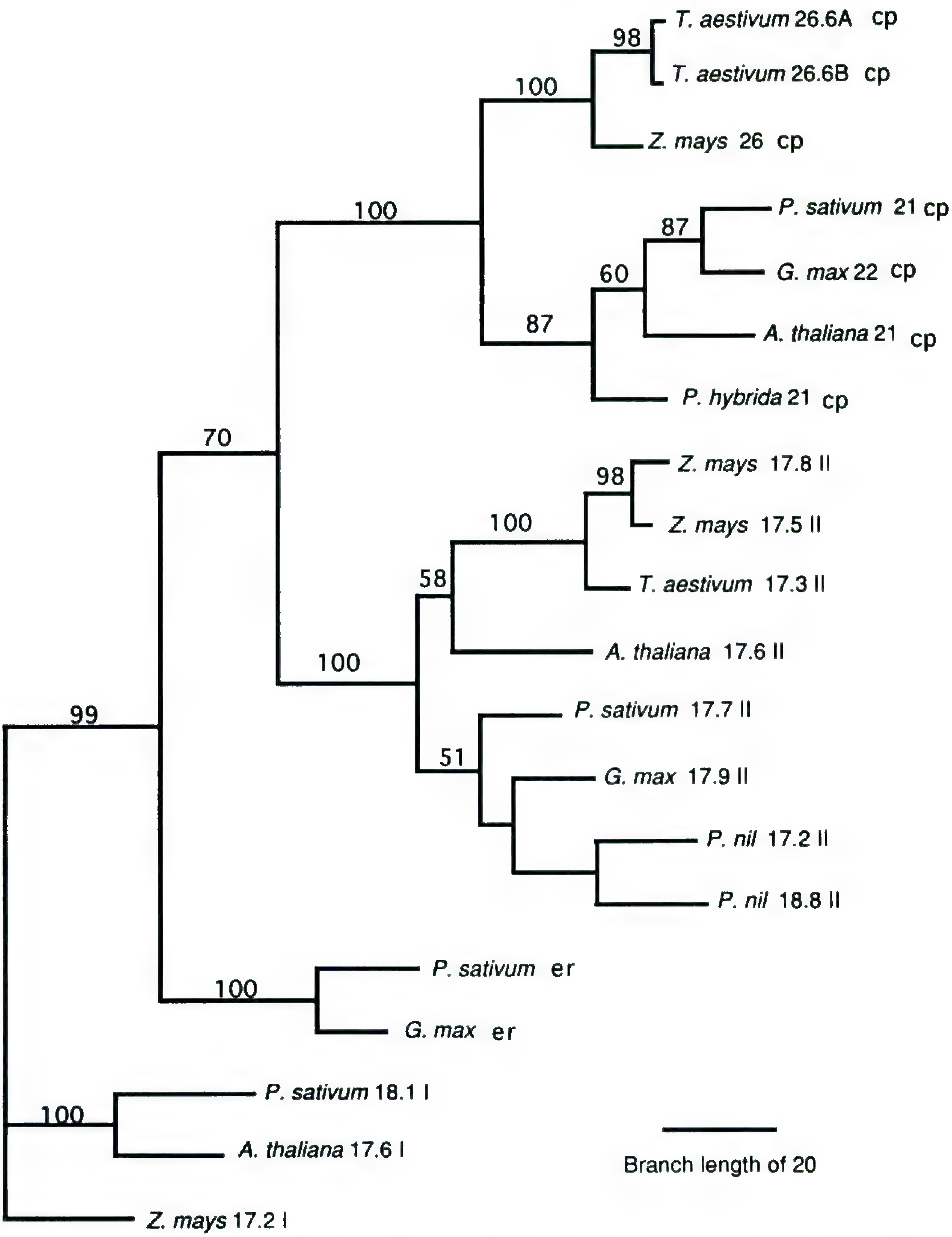

FIGURE 5. Tree of the small heat shock genes. The numbers above the branches are the numbers of times out of 100 bootstrap replicates that the branch was present. The names of the genes encoding the Class I cystosolic proteins are followed by I, those encoding the Class II cytosolic proteins by II, those encoding the endomembrane-localized proteins by er, and those encoding the chloroplast-localized proteins by cp.

of these sequences may prove informative in close comparisons.

For a gene to be useful in phylogenetic analysis the following four criteria need to be met. First, the gene should be present in a single copy within a genome. Second, there should be no variation below the species level but sufficient genetic variation between the species or taxa of interest. Third, if the gene is a member of a multi-gene family, orthologous and paralogous genes should be easily distinguishable. Fourth, the gene should have enough sequence similarity across the taxa of interest for Southern blot hybridization and primer annealing to be possible and no or few introns (Friendlander et al., 1992). In this paper, I have presented evidence that the small heat shock genes may be useful for phylogenetic analysis of plants. Restriction Fragment Length Polymorphism (RFLP) analysis indicates that the small heat shock genes analyzed in the Brassicaceae are single copy and are not variable below the species level but are variable between genera. The gene sequences of the four families of genes are readily distinguishable based on overall similarity comparisons and phylogenetic analysis. The sequences of the different gene families are sufficiently divergent (approximately 50%) that cross-hybridization in Southern blot analysis is unlikely, while within-family sequence conservation permits amplification of genes using PCR. However, not all the small heat shock genes may be useful. The orthologous and paralogous relationships among the genes for the Class II proteins may be complex. More sequences will be needed before this gene family can be fully evaluated for its utility in phylogenetic analysis.

DNA sequences for protein-coding nuclear genes may be very useful in inferring phylogenetic relationships, but they should not be used without careful examination of their evolutionary dynamics, including gene duplication and conversion. Of the four families of the small heat shock gene families, the genes coding for the chloroplast-localized protein are the most promising for phylogenetic study: a more detailed study of their usefulness in phylogenetic analysis is now in progress.

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                        1                                                50
T. aestivum 26.6A    CCTCGTCGCG CT---GGATT TGACATCTCC CCG------T TCGGGCTAGT
T. aestivum 26.6b    CCTCGTCGCG CT---GGATT TGACATCTCC CCG------T TCGGGCTAGT
P. sativum 21        CCTCGCAGAA GT---TCCAT CGATATTTCA CCA------T TTGGTTTATT
G. max 21            CCACGTAGAA CT---GCCAT GGACATTTCA CCA------T TTGGTATCTT
A. thaliana 21       CAACAACGCT TA---ACCAT GGACGTCTCT CCT------T TCGGATTGTT
P. hybrida 21        CCTAGGAGAA TG---GCTCT TGATGTCTCC CCT------T TCGGGTTATT
Z. mays 17.8         GACGCGGTGA TGTTCGGGCT GGAGACT--- ---------- --------CC
Z. mays 17.5         GACGGGAGGA TGTTCGGTCT CGAGACC--- ---------- --------CC
T. aestivum 17.3     GCGGGCATGG TGTTCGGCTT GGATGCC--- ---------- --------CC
P. sativum 17.7      GATTTCAGGC TAATGGATTT GGATTCT--- ---------- --------CC
G. max 17.9          GATTTCAGAG TGATGGGTTT GGAGTCT--- ---------- --------CC
P. nil 17.2          GATTTGAGGT TGATGGGTTT TGATCAC--- ---------- --------CC
P. nil 18.8          GATCTCAGGA ACTTTGGCTT ATCCAACTTC GGGTTAGAAC CG---CAGCT
A. thaliana 17.6II   GATTTAGGAA GGTTT----- ---------- ---------- ----------
P. sativum 18.8      TCTCTGATTC CAAGT----- -TTCTTTAGT ---GGCCGAA GG------AG
A. thaliana 17.6     TCTCTAATTC CAAGC----- -ATCTTCGGA ---GGAAGAA GA------AC
Z. mays 17.2         TCGCTCGTG- ---------- ---------- ------AGGC GC------AG
P. sativum er        GCTGATTTTC CTTTGAAAGC AAAAGGATCA CTACTACCAT TCATAGAT--
G. max er            ---------- -----AAAGC AAACGGGTCT CTGCTCCCAT TCATGGAT--

                     51                                                 100
T. aestivum 26.6A    GGACCCGATG TCGCCGATGA GGACGATGCG GCAGATGCTG GACACGATGG
T. aestivum 26.6B    GGACCCGATG TCGCCGATGA GGACGATGCG GCAGATGCTG GACACGATGG
P. sativum 21        GGACCCATGG TCACCTATGA GAAGCATGCG TCAGATGCTA GACACAATGG
G. max 21            GGACCCGTGG TCACCCATGA GGAGCATGCG CCAGATATTG GACACCATGG
A. thaliana 21       GGATCCTTTG TCACCAATGA GGACGATGCG ACAAATGTTA GATACTATGG
P. hybrida 21        AGACCCAATG TCTCCCATGA GAACAATGAG GCAGATGATG GACACTATGG
Z. mays 17.8         CCTGATGGCG GCGCTGCAGC ACCTGCTGGA CGTGCCCGAC GGCGACGCCG
Z. mays 17.5         CCTGATGGTG GCGCTGCAGC ACCTTCTGGA CGTGCCCGAC GGCGACGCCG
T. aestivum 17.3     GATGATGGCC GCCCTGCAGC ACCTGCTGGA CATCCCGGAC GGCGAGGCGG
P. sativum 17.7      ACTCTTCAAC ACTCTCCATC ATATAATGGA CCTCACCGAC GAC---ACAA
G. max 17.9          ACTGTTCCAC ACGCTGCAAC ACATGATGGA CATGTCAGAG GAC---GGTG
P. nil 17.2          GCTCTTC--- ------CACC ACATCATGGA CTACGCCGGC GAC---GACA
P. nil 18.8          CCTCTCAACC ATCCAAGACA TGCTCGACTT CGCCGACGAC CACGACAGAG
A. thaliana 17.6II   -CCAATAATC TCAATCCTCG AAGACATGCT TGAAGTCCCC GAAGACCACA
P. sativum 18.8      CAATGTTTTC GATCCTTTCT CCCTGGAC-- -GTCTGGGAT CCTTTGAAGG
A. thaliana 17.6     AAACGTTTTC GATCCGTTCT CGCTGGAT-- -GTTTTCGAT CCGTTCGAAG
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APPENDIX (pp. 289–295). DNA alignment of sequences listed in Table 1. The sequences coding for the transit peptides and the start ATG codons were removed and were not used in the analysis. A "-" denotes a gap in the alignment.

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Z. mays 17.2          CAACGTGTTC GACCCCTTCT CGATGGAC-- -CTCTGGGAT CCCTTCGACA
P. sativum er         -------TCT CCCAACACTC TCTTATCGGA TCTCTGGTCT GATCGTTTCC
G. max er             -------CCT CCCATCACTC TCTTGGCTGA TCTCTGGTCC GATCGCTTCC

                      101                                               150
T. aestivum 26.6A     ACCGGCTGTT CGACGACGCC GTGGGGTTCC CC------AC GCGTCGCTCG
T. aestivum 26.6B     ACCGGCTGTT CGACGACGCC GTGGGGTTCC CC------AC GGCGCGCTCG
P. sativum 21         ACAGAATTTT CGAAGATGCA ATCACAATTC CT---GGA-- -AGAAACATT
G. max 21             ACCGAGTTTT CGAAGATACC ATGACATTCC CT---GGA-- -AGAAACATA
A. thaliana 21        ACAGGATGTT CGAGGACACT ATGCCTGTCT CA---GGA-- -AGAAACAGA
P. hybrida 21         ACAGGCTATT CGAGGATACC ATGACATTCC CT---GGATC AAGGAACAGA
Z. mays 17.8          GCGCGGGTGG CGACAACAAG ACGGGCAGCG GCGGCAGCGC CACGCGCACC
Z. mays 17.5          GCGCGGGCGG CGACAAGGCG ------GGCG GCGGCGGGCC CACGCGCACC
T. aestivum 17.3      AGCCGCCGCC GGAGAAG--- ---------- --CAGGGCCC GACGCGCGCC
P. sativum 17.7       CCGAGAAGAA C--------- ---------T TAAACGCTCC AACTCGAACA
G. max 17.9           CAGGAGACAA CAAG------ ------ACAC ACAATGCTCC AACATGGTCA
P. nil 17.2           AGTCATCCAA CAGC------ ---------- --AGCGCGCC TTCTCGGACG
P. nil 18.8           CCGGCCGGGC C--------- ------CCAC CAGAACAACC CATCCGGGCC
A. thaliana 17.6II    ACAACGAGAA G--------- ------ACCC GCAACAACCC TTCACGAGTT
P. sativum 18.8       ACTTTCCATT TTCAAATTCT TCACCTTCCG CT---TCATT CCCT------
A. thaliana 17.6      GTTTCTTGAC GCCG---TCA GGATTGGCAA ACGCACCC-- -GCT------
Z. mays 17.2          CCATG---TT CCGCTCCATC GTCCCGTCGG CG-------- -ACCTCCACC
P. sativum er         CAGATCCGTT TCGCGTCTTA GAACAAATT- -----CCCTA TGGAGTTGAG
G. max er             CAGACCCGTT TCGCGTGCTG GAACATATT- -----CCGTT TGGGGTTGAC

                      151                                               200
T. aestivum 26.6A     CCAGCGGCGC GAGCGAGA-- ----CGCCGG ATGCCGTGGG ACATCATGGA
T. aestivum 26.6B     CCGGCGCGGC GAGCGAAGAC GCCG---CGG ATGCCGTGGG ACATTATGGA
P. sativum 21         GGAGGA---- -----GGTGA GATT---CGT GTGCCTTGGG AAATCAAAGA
G. max 21             GGAGGA---- -----GGGGA GATC---CGT GCCCCTTGGG ACATCAAAGA
A. thaliana 21        GGAGGAAGTG GAGTGTCAGA GATT---CGT GCACCGTGGG ACATCAAAGA
P. hybrida 21         GGAACA---- -----GGGGA AATA---CGT GCCCCTTGGG ACATCAAAGA
Z. mays 17.8          TACGTCCGCG ACGCGCGCGC CATGGCGGCC ACCCCGGCCG ACGTGAAGGA
Z. mays 17.5          TACGTCGCCG ACGCGCGCGC CATGGCGGTC ACCCCGGCCG ACGTCAAGGA
T. aestivum 17.3      TACGTCCGCG ACGCGCGCGC CATGGCGGCC ACCCCCGCCG ACGTGAAGGA
P. sativum 17.7       TATGTCCGTG ACGCAAAGGC AATGGCTGCA ACTCCAGCGG ACGTGAAAGA
G. max 17.9           TACGTTCGAG ACGCGAAAGC AATGGCTGCA ACACCTGCGG ATGTGAAGGA
P. nil 17.2           TTTATGCTGG ACGCCAAGGC AATGGCAGCC ACCCCGGCGG ACGTGAAGGA
P. nil 18.8           TACGTCCGGG ACGCGAAGGC GATGGCAGCG ACCCCGGCGG ACGTGAAGGA
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APPENDIX. Continued.

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A. thaliana 17.6II   TACATGCGAG ACGCTAAGGC AATGGCTGCT ACACCTGCTG ACGTCATCGA
P. sativum 18.8      ------CGTG AGAATCCTGC TTTTGTGAGC ACACGAGTTG ACTGGAAGGA
A. thaliana 17.6     ------ATGG ATGTGGCAGC GTTCACAAAC GCTAAAGTGG ATTGGAGGGA
Z. mays 17.2         AAC---TCCG AGACTGCCGC CTTCGCCAGC GCCCGCATCG ACTGGAAGGA
P. sativum er        AAACACGAAC CATCCATAAC ATTGTCACAT GCTAGAGTAG ACTGGAAGGA
G. max er            AAAGATGAAG CATCCATGGC CATGTCACCT GCTAGAGTGG ACTGGAAGGA

                  201                                                250
T. aestivum 26.6A    GGACGAGAAG GAGGTGAAGA TGCGGTTTGA CATGCCTGGG CTGTCGCGGG
T. aestivum 26.6B    GGACGAGAAG GAGGTGAAGA TGCGGTTTGA CATGCCTGGG CTGTCGCGGG
P. sativum 21        TGAAGAACAT GAAATCAGAA TGCGTTTTGA TATGCCTGGT GTTTCTAAGG
G. max 21            TGAAGAACAT GAAATCAGAA TGAGGTTTGA CATGCCGGGT CTTGCCAAGG
A. thaliana 21       GGAAGAACAC GAGATCAAGA TGCGTTTCGA CATGCCTGGT CTCTCTAAAG
P. hybrida 21        CGATGAAAAT GAAATCAAGA TGCGTTTTGA TATGCCAGGG CTATCTAAAG
Z. mays 17.8         GCTGCCCGGC GCGTACGCGT TCGTGGTGGA CATGCCGGGG CTGGGCACGG
Z. mays 17.5         GCTCCCGGGC GCGTACGCGT TCGTGGTGGA CATGCCGGGG CTGGGCACGG
T. aestivum 17.3     GCTGCCGGGC GCGTACGCGT TCGTGGTGGA CATGCCGGGG CTCGGGTCCG
P. sativum 17.7      GCATCCAAAT TCATACGTGT TTATGGTGGA CATGCCTGGG GTGAAATCTG
G. max 17.9          GTATCCGAAT TCTTACGTGT TCGAGATCGA CATGCCGGGT TTGAAATCTG
P. nil 17.2          GTACCCAAAC TCCTACGTCT TCATCATAGA CATGCCGGGG CTGAAGTCCG
P. nil 18.8          GTATCCGAAT TCCTACGTGT TCATCGCGGA CATGCCGGGG GTAAAGGCCG
A. thaliana 17.6II   GCACCCTAAC GCATATGCAT TCGTCGTGGA CATGCCTGGA ATCAAAGGAG
P. sativum 18.8      AACACCGGAA GCGCATGTTT TCAAGGCTGA TCTTCCTGGG CTGAAAAAGG
A. thaliana 17.6     GACACCTGAG GCGCACGTGT TCAAGGCGGA TTTACCGGGA CTGAGGAAGG
Z. mays 17.2         GACGCCCGAG GCGCACGTCT TCAAGGCCGA CCTCCCCGGC GTCAAGAAGG
P. sativum er        AACTCCAGAG GGACATGTGA TAATGGTGGA CGTGCCTGGG TTGAAAAAAG
G. max er            GACCCCAGAG GGGCATGTTA TAATGCTGGA CGTGCCGGGG CTGAAGAGAG
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                     251                                                300
T. aestivum 26.6A    AGGAGGTGAG GGTGATGGTG GAGGACGACG CGCTGGTCAT CCGCGGCGAG
T. aestivum 26.6B    AGGAGGTGAG GGTGATGGTG GAGGACGACG CGCTGGTCAT CCGCGGCGAG
P. sativum 21        AAGACGTTAA AGTATCAGTT GAAGATGATG TTCTTGTCAT AAAAAGTGAT
G. max 21            AAGATGTTAA GGTATCAGTG GAGGATGATA TGCTTGTTAT AAAAGGTGGC
A. thaliana 21       AAGACGTCAA AATCTCTGTA GAAGATAACG TACTTGTGAT CAAAGGAGAG
P. hybrida 21        AAGAGGTAAA AGTATCAGTG GAAGATGATG TGCTTGTCAT CAAAGGTGAA
Z. mays 17.8         GCGACATCCG GGTGCAGGTG GAGGACGAGC GGGTGCTGGT GGTCAGCGGC
Z. mays 17.5         GCGACATCAA GGTGCAGGTG GAGGACGAGC GGGTGCTGGT GATCAGCGGC
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APPENDIX. Continued.

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T. aestivum 17.3     GCGACATCAA GGTGCAGGTG GAGGACGAGC GGGTGCTGGT GATCAGCGGC
P. sativum 17.7      GTGACATAAA GGTTCAGGTG GAAGATGAGA ATGTGCTATT GATAAGTGGC
G. max 17.9          GGGACATAAA GGTTCAAGTT GAAGACGACA ACCTGCTTCT GATATGTGGG
P. nil 17.2          GCGACATCAA GGTGCAGGTC GACGGCGACA ACGTGTTGAG TATTAGCGGG
P. nil 18.8          CGGAAATCAA GGTCCAGGTG GAGGACGACA ACGTTTTGGT GGTGAGCGGG
A. thaliana 17.6II   ATGAGATCAA GGTTCAGGTC GAGAACGACA ATGTGCTTGT GGTGAGTGGA
P. sativum 18.8      AGGAAGTGAA AGTTGAAGTT GAAGATGATA GGGTTCTACA GATAAGCGGA
A. thaliana 17.6     AAGAGGTGAA AGTGGAGGTT GAGGATGGCA ACATACTTCA GATAAGTGGA
Z. mays 17.2         AGGAGGTCAA GGTTGAGGTC GAAGACGGCA ACGTGCTGGT CATCAGCGGC
P. sativum er        ATGATATAAA GATAGAAGTG GAAGAGAATA GGGTGCTAAG AGTGAGTGGT
G. max er            AAGAGATAAA GGTAGAGGTG GAAGAGAATA GGGTGCTGAG AGTGAGTGGT

                     301                                                  350
T. aestivum 26.6A    CACAAGAAGG AG------GC CGGCGAAGGG CAGGGCGAAG GCGGCGACGG
T. aestivum 26.6B    CACAAGAAGG AG------GC CGGCGAAGGG CAGGGCGAAG GCGGCGACGG
P. sativum 21        CATAGA---- ---------- -GAGGAAAAT GGTGGAGAAG AT--------
G. max 21            CACAAAAGTG AA-------- -CAAGAACAT GGTGGAGATG AT--------
A. thaliana 21       CAGAAGAAGG AA-------- ---------- ---------G ACAGTGATGA
P. hybrida 21        CACAAAAAGG AA-------- ---------- ------AGTG GAAAGGATGA
Z. mays 17.8         GAGCGGCGCC GGGAG----- ----GAGCGC GAG---GACG ACGCCAAGTA
Z. mays 17.5         GAGCGGCGCC GGGAG----- ----GAGCGC GAG------G ACGCCAAGTA
T. aestivum 17.3     GAGCGGCGGA GGGAG----- ----GAGAAG GAG------G ACGCCAAGTA
P. sativum 17.7      GAGAGG---A AGAGA----- ----GAAGAA GAGAAAGAAG GTGTTAAATA
G. max 17.9          GAACGA---A AGAGG----- ----GACGAA GAGAAAGAAG GGGCGAAGTA
P. nil 17.2          GAGCGG---A AGCGT----- -GAGGCGGAG GAGAAGGAGG GGGCGAAGTA
P. nil 18.8          GAGCGAACGG AGAGG----- -GAGAAAGAC GAGAAGGATG GGGTGAAGTA
A. thaliana 17.6II   GAGAGGCAGA GAGAG----- -AACAAAGAA AAC---GAAG GTGTGAAGTA
P. sativum 18.8      GAGAGAAGCG TTGAG----- ----AAAGAA GAT---AAGA ATGATGAATG
A. thaliana 17.6     GAGAGGAGCA ATGAG----- ----AATGAA GAG---AAGA ATGACAAGTG
Z. mays 17.2         CAGCGCAGCA GGGAG----- ----AAGGAG GAC---AAGG ACGACAAGTG
P. sativum er        GAGAGGAAGA AAGAA----- ----GAAGAT AAA---AAAG GAGATCATTG
G. max er            GAAAGGAAGA AGGAG----- ----GAGGAG AAG---AAGG GGGATCACTG

                     351                                                  400
T. aestivum 26.6A    GTGGTGGAAG GAGCGCAGCG TGAGCTCCTA CGACATGCGC CTTGCTCTG-
T. aestivum 26.6B    GTGGTGGAAG GAGCGCAGCT TGAGCTCCTA CGACATGCGA CTGGCTCTG-
P. sativum 21        -TGTTGGTCA AGAAAGAGTT ATAGTTGTTA TGATACTCGT CTCAAGCTT-
G. max 21            -TCTTGGTCA AGCAGGACCT ATAGTTCCTA TGATACCCGT TTAAAGCTG-
P. hybrida 21        CTCGTGGGGA AGG---AATT ACAGTTCCTA TGACACTCGT CTAAGTTTA-
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APPENDIX. Continued.

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Z. mays 17.8         CCTGCGCATG GAGCGGCGGA TGGGCAAGTT CATGCGCAAG TTCGTGCTG-
Z. mays 17.5         CCTGCGCATG GAGCGGCGGA TGGGCAAGTT CATGCGCAAG TTCGTGCTG-
T. aestivum 17.3     CCTGCGGATG GAGCGCCGCA TGGGCAAGCT GATGCGCAAG TTCGTGCTG-
P. sativum 17.7      TTTGAAGATG GAAAGAAGGA TTGGTAAGTT GATGAGGAAA TTTGTGTTA-
G. max 17.9          TTTGAGAATG GAGAGAAGGG TTGGGAAGTT AATGCGCAAG TTTGTGCTG-
P. nil 17.2          TGTGAGGATG GAGAGGAGGG TGGGGAAGTT AATGAGGAAG TTTGTGCTG-
P. nil 18.8          TTTGAGGATG GAACGGAGGG TGGGGAAGTT TATGAGGAAA TTTGTGCTG-
A. thaliana 17.6II   TGTGAGGATG GAGAGAAGAA TGGGTAAGTT CATGAGGAAG TTTCAGTTG-
P. sativum 18.8      GCATCGCGTG GAACGTAGCA GTGGAAAGTT CTTAAGAAGG TTCAGATTG-
A. thaliana 17.6     GCACCGTGTG GAGCGGTCGA GTGGGAAGTT CACGAGGAGG TTTAGGCTG-
Z. mays 17.2         GCACCGTGTC GAGCGCAGCA GTGGCCAGTT CATCAGGCGC TTCCGCCTG-
P. sativum er        GCACAGAGTT GAAAGATCTT ATGGAAAGTT CTGGAGGCAG TTTAAATTA-
G. max er            GCACAGAGTG GAGAGATCCT ATGGCAAGTT CTGGAGGCAG TTCAGGTTG-

                  401                                                    450
T. aestivum 26.6A    --CCGGACGA GTGCGACAAG AGCCAGGTGC GGGCCGAGCT CAAGAACGGC
T. aestivum 26.6B    --CCGGACGA GTGCGACAAG AGCCAGGTGC GCGCCGAGCT CAAGAACGGC
P. sativum 21        --CCTGATAA CTGTGAGAAA GAGAAGGTTA AGGCTGAGTT GAAAGATGGT
G. max 21            --CCGGATAA TTGCGAGAAG GACAAGGTTA AGGCAGAGTT GAAAAATGGT
A. thaliana 21       --CCAGACAA CTGTGAGAAA GACAAGATCA AAGCTGAGCT CAAGAACGGA
P. hybrida 21        --CCTGATAA TGTTGACAAA GATAAAGTTA AAGCAGAATT GAAGAATGGA
Z. mays 17.8         --CCGGACAA CGCCGACGTC GACAAGGTCG CCGCCGTGTG CAGGGACGGC
Z. mays 17.5         --CCGGACAA CGCCGACATG GACAAGATCT CGGCGGTGTG CAGGGACGGC
T. aestivum 17.3     --CCGGAGAA CGCCGACATG GAGAAGATCT CGCCG---TG CCGCGACGGC
P. sativum 17.7      --CCTGAGAA TGCGAATATT GAAGCTATCT CTGCTATTTC TCAAGATGGT
G. max 17.9          --CCTGAGAA TGCCAACACT GATGCAATCT CTGCTGTGTG CCAAGATGGT
P. nil 17.2          --CCGGAAAA TGCGAATAAG GAGAAGATCA CGGCGGTTTG CCAGGATGGG
P. nil 18.8          --CCGGAGAA TGCCAACGTG GAGGCGATAA ATGCGGTTTA TCAGGACGGG
A. thaliana 17.6II   --CCTGAAAA TGCAGATTTG GACAAGATCT CTGCTGTTTG TCATGACGGT
P. sativum 18.8      --CCTGAGAA TGCTAAAATG GATAAAGTGA AAGCTTCCAT GGAGAACGGC
A. thaliana 17.6     --CCGGAGAA TGCTAAGATG GAGGAAATCA AAGCCAGTAT GGAAAATGGT
Z. mays 17.2         --CCGGATGA CGCCAAGGTG GATCAGGTCA AGGCTGGCCT CGAGAACGGC
P. sativum er        --CCTCAAAA TGTTGATTTG GATTCTGTCA AAGCTAAAAT GGAAAACGGT
G. max er            --CCACAAAA TGTAGACTTG GATTCTGTCA AGGCCAAGCT GGAGAATGGG

                     451                                                    500
T. aestivum 26.6A    GTGCTGCTCG TGTCCGTGCC CAAGAGG--- ---------- ----------
T. aestivum 26.6B    GTGCTGCTCG TGTCCGTGCC CAAGAGG--- ---------- ----------
P. sativum 21        GTGCTTTATA TTACTATTCC TAAGACT--- ---------- ----------
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APPENDIX. Continued.

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G. max 21            GTGCTTTATA TCACCATTCC TAAGACC--- ---------- ----------
A. thaliana 21       GTCCTCTTTA TCACTATCCC TAAGACC--- ---------- ----------
P. hybrida 21        GTTCTTTTAA TCTCAATTCC TAAGACC--- ---------- ----------
Z. mays 17.8         GTGCTCACGG TGACCGTTGA GAAGCTGCCC ---------- ----------
Z. mays 17.5         GTGCTCACCG TGACCGTCGA GAAGCTGCCC ---------- ----------
T. aestivum 17.3     GTGCTCACCG TGACCGTCGA CAAGCTGCCG ---------- ----------
P. sativum 17.7      GTTCTTACGG TTACAGTTAA TAAATTGCCT ---------- ----------
G. max 17.9          GTGCTTAGTG TAACCGTGCA GAAATTGCCT ---------- ----------
P. nil 17.2          GTGTTGACGG TGACGGTGGA GAACGTGCCG ---------- ----------
P. nil 18.8          GTGTTGCAGG TGACGGTGGA GAAATTGCCG ---------- ----------
A. thaliana 17.6II   GTGTTGAAGG TTACTGTTCA GAAACTTCCT ---------- ----------
P. sativum 18.8      GTTCTGACAG TGACCGTTCC AAAA---GAA GAG------- ----------
A. thaliana 17.6     GTGTTGTCGG TTACCGTGCC CAAG---GTG CCA------- ----------
Z. mays 17.2         GTGCTCACGG TCACCGTGCC TAAG---GCG ---------- ----------
P. sativum er        GTTCTTACTT TAACTCTTCA TAAGTTGTCG CATGATAAGA TTAAAGGTCC
G. max er            GTGCTCACTT TGACACTTGA CAAGTTGTCA CCTGGTAAGA TCAAAGGTCC

                     501                                                550
T. aestivum 26.6A    ---------- ---------- --------GA GACCGAGCGC AAGGTCATCG
T. aestivum 26.6B    ---------- ---------- --------GA GACCGAGCGC AAGGTCATCG
P. sativum 21        ---------- ---------- --------AA AATTGAACGC ACGGTTATTG
G. max 21            ---------- ---------- --------AA GGTTGAACGT AAGGTTATTG
A. thaliana 21       ---------- ---------- --------AA AGTCGAACGC AAAGTCATCG
P. hybrida 21        ---------- ---------- --------AA AGTTGAGAAG AAGGTGACTG
Z. mays 17.8         ---------- ---------- -----CCGCC GGAGCCCAAG AAGCCCAAGA
Z. mays 17.5         ---------- ---------- -----CCGCC CGAGCCCAAG AAGCCCAAGA
T. aestivum 17.3     ---------- ---------- -----CCGCC CGAGCCCAAG AAGCCCAAGA
P. sativum 17.7      ---------- ---------- -----CCACC TGAACCTAAG AAACCAAAAA
G. max 17.9          ---------- ---------- -----CCACC TGAGCCTAAG AAACCTAGGA
P. nil 17.2          ---------- ---------- -----CCGCC GGAACCGAAG AAGCCAAGGA
P. nil 18.8          ---------- ---------- -----CCGCC GGAGCCGAAG AAGCCAAAGA
A. thaliana 17.6II   ---------- ---------- -----CCTCC GGAACCAAAG AAACCAAAGA
P. sativum 18.8      ---------- ---------- -----ATAAA GAAGGCTGAG GTTAAGTCTA
A. thaliana 17.6     ---------- ---------- -----GAGAA AAAGCCAGAG GTTAAGTCCA
Z. mays 17.2         ---------- ---------- -----GAAGA GAAGAAGCCT GAGGTGAAGG
P. sativum er        TAGAATGGTT AGTATTGTGG AAGAGGATGA CAAACCATCT AAGATCGTCA
G. max er            CAGGGTGGTC AGCATTGCTG GGGAGGATCA CCAACAAGGT AATCTCAACA

                     551                    573
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APPENDIX. Continued.

T. aestivum 26.6A	ACGTGCAGGT	CCAG------	---
T. aestivum 26.6B	ACGTGCAGGT	CCAG------	---
P. sativum 21	ATGTTCAAAT	TCAG------	---
G. max 21	ATGTCCAAGT	TCAG------	---
A. thaliana 21	ATGTCCAGAT	TCAG------	---
P. hybrida 21	ACGTGGAA--	----------	---
Z. mays 17.8	CCATCGAGGT	CAAGGTCGCC	---
Z. mays 17.5	CCATCGAGGT	CAAGGTCGCC	---
T. aestivum 17.3	CCATCCAGGT	CCAGGTCGCC	---
P. sativum 17.7	CTATTCAAGT	TAAGGTTGCT	---
G. max 17.9	CTATTCAGGT	TAAGGTTGCT	---
P. nil 17.2	CCATTGAGGT	CAAAATTGGT	---
P. nil 18.8	CAGTTGAAGT	CAAGGTTGCG	---
A. thaliana 17.6II	CAATTCAAGT	TCAAGTTGCT	---
P. sativum 18.8	TTGAGATTTC	TGGT------	---
A. thaliana 17.6	TTGATATCTC	TGGT------	---
Z. mays 17.2	CTATTGAGAT	CTCTGGT---	---
P. sativum er	ATGATGAGTT	GAAA------	---
G. max er	ATGATGGGGC	CAAGCAGGAG	CTT

APPENDIX. Continued.

EVOLUTION OF THE PHYTOCHROME GENE FAMILY AND ITS UTILITY FOR PHYLOGENETIC ANALYSES OF ANGIOSPERMS[1]

Sarah Mathews, Matt Lavin, and Robert A. Sharrock[2]

Abstract

The phytochrome gene family encodes photoreceptor proteins that serve many functions throughout the life of a plant. From studies of the angiosperm *Arabidopsis*, the family has been modeled as comprising five loci, *PHYA–PHYE*. However, in most nonangiosperms, one locus, or at most two, is present. Moreover, it is shown here that the *Arabidopsis* model does not completely represent some angiosperm groups. For example, additional *PHY* loci related to *PHYA* and *PHYB* of *Arabidopsis* have evolved independently several times in dicot angiosperms, and monocot angiosperms (as well as *Piper*) may lack orthologs of *Arabidopsis PHYD* and *PHYE*. Nonetheless, for studies of organismal evolution, the phytochrome gene family is a potential source of phylogenetic information because the loci occur as single copy sequences, and preliminary data suggest that the various loci are evolving independently. In the plant family Fabaceae, phytochrome data are shown to provide phylogenetic resolution to a taxonomically very difficult tribe of tropical woody genera that include *Millettia, Lonchocarpus*, and *Derris*. In addition to nucleotide substitutions, phylogenetically informative insertions and deletions helped to resolve relationships in this group of legumes. Also, the presence of a legume-specific locus related to *PHYA* should prove to be phylogenetically informative once its taxonomic distribution is better understood.

Most molecular phylogenies of plants are inferred from one or two genes, and these usually from chloroplast or nuclear ribosomal DNA sequences. When discordance between molecular phylogenies occurs, biological phenomena such as introgressive hybridization or lineage sorting from polymorphic ancestry may explain the disparity (e.g., Harrison et al., 1987; Rieseberg & Brunsfeld, 1992; Soltis et al., 1992). Such differences also may result from lack of resolution in one of the data sets (e.g., Olmstead, 1989), or from mistaken orthology (e.g., Goodman et al., 1979; Doyle, 1992). Thus, determining organismal relationships requires that evolutionary hypotheses derived from single genes be tested with further data (e.g., Pamilo & Nei, 1988; Takahata, 1989). DNA sequences from the low copy fraction of the nuclear genome potentially provide novel phylogenetic resolution, specifically at the organismal level, since certain of the processes that lead to incongruence of species and gene trees (e.g., uniparental inheritance, nonhomologous recombination) may be less frequent.

The low copy fraction of nuclear DNA remains underexplored in phylogenetic studies of plants, and initial investigations of DNA sequences from multigene families have revealed some potential problems related to concerted evolution (sensu Zimmer et al., 1980). For example, an analysis of *rbc*S nucleotide sequences (Meagher et al., 1989) indicated that gene conversions among *rbc*S loci have occurred in each genus examined, leading to regions of "partial homology" (Patterson, 1987) and thus to the possibility of mistaken orthology. Sanderson & Doyle (1992) suggested, however, that the probability of reconstructing a reliable organismal phylogeny is high from DNA sequences of multigene families in which concerted evolution is infrequent. Preliminary data indicate that this is the case in such gene families as actin (Shah et al., 1983; Drouin & Dover, 1990; McElroy et al., 1990) and phytochrome (Sharrock & Quail, 1989; Dehesh et

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al., 1991; Heyer & Gatz, 1992a, b; Clack et al., 1994; Adam et al., 1993); consequently, these multigene families should yield data pertinent to studies of organismal phylogenies. Furthermore, an advantage of multigene families in phylogenetic reconstruction is that, in addition to nucleotide substitution and insertion/deletion characters, the presence or absence of loci can be phylogenetically informative.

The phytochromes are photoreceptors for red and far-red light in all land plants and green algae (reviewed in Quail, 1991; Furuya, 1993). Each subunit of these large cytoplasmic receptors comprises a protein of 1100 to 1200 amino acids and a covalently attached linear tetrapyrrole chromophore. Existing in two continuously interconvertible forms, Pr, the red light-absorbing form, and Pfr, the far-red light-absorbing and biologically active form, phytochrome mediates diverse developmental responses throughout the plant's life cycle. These responses include germination, seedling hypocotyl elongation, stem cell differentiation, plastid development, flavonoid pigment synthesis, and floral induction in response to photoperiod. Modulation of plant gene expression by phytochrome is well documented (Nagy et al., 1988). While the mechanisms whereby phytochrome participates in cellular signalling remain unknown, regions of the polypeptide required for chromophore attachment, spectral integrity, biological activity, and dimerization have been identified (Cherry et al., 1993; Edgerton & Jones, 1992).

Several reports have described the presence of only a single *PHY* gene in certain nonangiosperms (Hanelt et al., 1992; Kolukisaoglu et al., 1993; Morand et al., 1993; Okamoto et al., 1993; Thümmler et al., 1992; Winands et al., 1992), while evidence of two *PHY* genes is reported for other nonangiosperms. For example, Maucher et al. (1992) refer to a putative second gene in the fern *Dryopteris filix-mas* L., although the fragment remains uncharacterized. Two unpublished *PHY* sequence fragments from *Psilotum nudum* (L.) Griseb. (GenBank accessions X74930, X74931) differ from one another in the region of overlap; and two *PHY* cDNAs from *Pinus palustris* Mill. reportedly have been cloned and partially sequenced (Furuya, 1993), while a single *PHY* cDNA from *Gingko biloba* L. is cited in the same report. However, in angiosperms, five related sequences encoding phytochrome proteins designated *PHYA–PHYE* have been characterized from *Arabidopsis thaliana* (D.C.) Schur (Sharrock & Quail, 1989; Clack et al., 1994). The genes for these five phytochromes have been mapped to *Arabidopsis* chromosomes 1, 2, 4, and 5 (unpublished), and no evidence for *PHY* pseudogenes was found. Homologs of *Arabidopsis PHYA* and *PHYB* have been characterized in other angiosperms (Adam et al., 1993; Christensen & Quail, 1989; Dehesh et al., 1991; Hershey et al., 1985; Heyer & Gatz, 1992a, b; Kay et al., 1989; Sato, 1988; Sharrock et al., 1986). A putative pseudogene most similar to *PHYA* has been reported in *Pisum* (Sato, 1990), and a cDNA clone from *Zea* containing a partial *PHY* fragment has been interpreted as a pseudogene (Christensen & Quail, 1989). Overall, these studies suggest that the gene family increases in complexity from nonangiosperms to angiosperms. This suggestion is consistent with data recently submitted to GenBank (see Results).

Nearly all *PHY* genes that are fully characterized share high sequence identity (App. 1) and structural similarity with the *Arabidopsis* loci (Fig. 1). Peptide fragments from the nonangiosperms *Psilotum* (Hanelt et al., 1992), *Anemia phyllitidis* (L.) Sw., and *Dryopteris filix-mas* (Maucher et al., 1992) share high sequence identity with the *Arabidopsis* phytochromes in their N-termini (App. 1, 2), and small internal *PHY* peptides from the alga *Mesotaenium caldariorium* (Lagerh.) Hansg. are highly similar to both N- and C-terminal peptides of other phytochromes (Morand et al., 1993). Two exceptional *PHY* genes have been described in nonangiosperms. The *PHY* gene sequence from the alga *Mougeotia scalaris* Hässel (Winands et al., 1992) contains additional introns in the N-terminal coding sequence, and in the *PHY* gene from the moss *Ceratodon purpureus* (Hedw.) Brid. the conserved N-terminal region is combined with a highly divergent C-terminal coding region (Fig. 1), which encodes a putative light-regulated protein kinase (Thümmler et al., 1992). However, in another moss, *Physcomitrella patens* (Hedw.) B.S.G., the C-terminal coding region is similar to all other *PHY* genes (Kolukisaoglu et al., 1993). No unusual *PHY* loci have been described in angiosperms.

The *PHYA–E* genes in *Arabidopsis* are differentially expressed in response to the light environment (Sharrock & Quail, 1989; Somers et al., 1991; Clack et al., 1994), and unique physiological functions have been assigned to two phytochrome proteins. Phytochrome A controls the far-red high-irradiance response (Nagatani et al., 1993; Parks & Quail, 1993; Whitelam et al., 1993), whereas phytochrome B controls red light regulation of stem length and flowering time, and the end-of-day far-red light response (Reed et al., 1993; Wester et al., 1994). This functional divergence together with high sequence divergence (approximately 50%

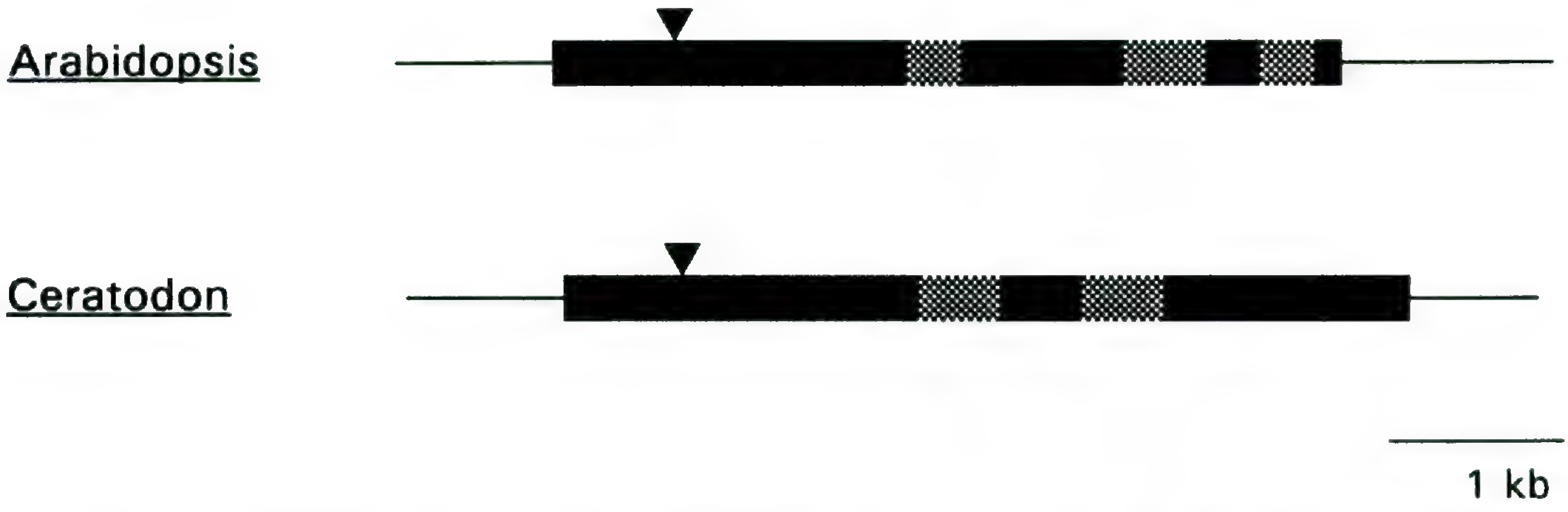

FIGURE 1. Phytochrome gene structure of *Arabidopsis* (Clack et al., 1994) and *Ceratodon* (Thümmler et al., 1992), from N-terminus (left) to C-terminus (right) showing untranslated regions (lines), exons (filled rectangles), introns (shaded rectangles), and the approximate site of chromophore attachment (triangle).

among the *PHYA*, *PHYB*, and *PHYC* loci) suggests that nonhomologous recombination is infrequent among *PHY* genes of *Arabidopsis*. If the loci are evolving independently, distinguishing orthologs from paralogs should not be difficult. To test this hypothesis, and to ascertain the phylogenetic utility of *PHY* sequence data, PCR (polymerase chain reaction) was used to sample multiple *PHY* loci from genomic DNAs of diverse species of land plants for sequence information, and these data were subjected to phylogenetic analysis.

MATERIALS AND METHODS

Total DNA was isolated from fresh, lyophilized, or dried herbarium material of taxa listed in Appendix 3 by standard methods (Doyle & Doyle, 1987). Aliquots were extracted once with phenol : chloroform-isoamyl alcohol (1 : 1 volume), and the aqueous portions were purified over sepharose CL-6B (Pharmacia, Piscataway, New Jersey) columns. To assess phytochrome diversity in early land plants, DNA sequences from different nonangiosperm phyla available in the literature (Appendix 2 and Kolukisaoglu et al., 1993) were included in the analyses with those determined during the present study. The most complete *PHY* sequence from *Psilotum* obtained from GenBank (accession X74931, lacking 510 3′ nucleotide sites out of the 3417 nucleotide sites in the full-length sequence data set) was used in phylogenetic analyses, but was not included in final alignments because it did not significantly affect the consensus sequence. Likewise, the *PHY* sequences from *Physcomitrella* and from the angiosperm *Nicotiana* (GenBank accessions X66784, L10114), were used in phylogenetic analyses, but were not included in Appendix 1. DNAs were sampled from different subclasses of angiosperms (sensu Cronquist, 1981) and, from legumes, DNAs were sampled to include two to three divergent members of the tribes Robinieae, Millettieae, and Dalbergieae in order to make preliminary evaluation of biogeographic hypotheses (e.g., Lavin & Luckow, 1993). The two species sampled from *Millettia* (*M. dura* Dunn and *M. richardiana* (Baill.) D. J. Du Puy & J. Labat) and *Sesbania* (*S. sesban* (L.) Morr. and *S. vesicaria* (Jacq.) Elliot) are not thought to be closely related within each genus.

A region of the *PHY* gene that encodes a peptide including and proximal to the chromophore attachment site was amplified using PCR, resulting in a target of 270–350 bp (App. 1). Oligonucleotides with equimolar mixtures of nucleotide pairs at two-fold degenerate sites and inosines (I) at three- to four-fold degenerate sites were designed to amplify all possible target sequences in template DNAs flanked by the conserved upstream peptide HYPATDIP (5′-CA[TC]TA[TC][TC]CIGCIACIGA[TC]AT[TCA]CC-3′) and downstream PFPLRYAC (5′-C[AG]CAIGC[AG]TAIC[GT]IA[AG]IGG[AG][AT]AIGG-3′). These peptide sequences are conserved in all *Arabidopsis* phytochromes and in the amino acid sequences inferred from other fully sequenced dicot and monocot genes, and they flank a region comprising variation likely to be phylogenetically informative. Standard PCR protocols (Perkin-Elmer, Norwalk, Connecticut) were modified to include an initial 5 cycles in which annealing temperatures were less stringent (e.g., 45–49°C). The PCR products were converted to blunt-end fragments with T4 DNA polymerase (BRL, Gaithersburg, Maryland) and were ligated to *Eco*RV-cut bacteriophage M13KRV8.2. M13KRV8.2 carries an *Eco*K cassette that facilitates screening of nonrecombinants in an *E. coli* strain which is $r_k^+m_k^+$

(Waye et al., 1985). Transformation of *E. coli* with the ligation product yielded a population of M13*PHY* clones containing amplified genomic *PHY* sequences. Individual clones were cultured, and double-stranded phage DNA was isolated from bacterial pellets by alkaline-lysis minipreparation. Inserts cut from M13 vectors using *Eco*RI and *Hind*III were resolved on 3% NuSieve (FMC, Rockland, Maine), or 2% standard, agarose gels, and in some cases were further screened by restriction enzyme digestion to avoid sequencing duplicate clones. Single-stranded DNAs for Sanger dideoxy sequencing (Sequenase version 2.0, USB, Cleveland, Ohio) were isolated from recombinants carrying putative *PHY* inserts. In most cases, sequences of both orientations were determined, and multiple PCR products from two accessions or genera were sequenced to detect possible contamination and PCR errors. Peptide sequences were multiply aligned using ALIGN (Scientific & Education Software, State Line, Pennsylvania) and GDE 2.2 (Steven Smith and University of Illinois) and were adjusted by eye; peptide alignments were the basis for multiple nucleotide sequence alignments. For sequence comparisons, alignment gaps in certain regions of insertion/deletion were deleted, while gaps that could be identified as homologous were coded as single characters. Nonhomologous 3′ and 5′ nucleotide sites were not included in the data matrices used in cladistic and distance analyses.

Sequences were compared using maximum parsimony algorithms available in PHYLIP (Felsenstein, 1993), Hennig86 (Farris, 1988), and PAUP (Swofford, 1993). Minimal length trees resulting from heuristic search options available in either Hennig86 (mh*, bb* with no upper limit set), PHYLIP (DNAPARS), or in PAUP (CLOSEST or RANDOM data addition sequence, HOLD option set for 5 trees when applicable, STEEPEST DESCENT, MULPARS, and TBR branch swapping options activated, with branch swapping on nonminimal trees, and MAXTREES set at 10,000) were used as starting trees for further PAUP analyses (CLOSEST data addition sequence, STEEPEST DESCENT, MULPARS and TBR options activated, with branch swapping on nonminimal trees), with the latter resulting in shorter trees. Support for monophyly of clades was evaluated using bootstrap resampling (Felsenstein, 1985) and decay analysis (Bremer, 1988). Pairwise distances were estimated using the Kimura 2-parameter option available in MEGA (Kumar et al., 1993) and absolute and relative evolutionary rates were calculated by the methods of Kimura (1981) and Wu & Li (1985) respectively. All matrices subject to distance, cladistic, and rate analyses are available on request from the first author. Tree analysis and graphical output were performed with MacClade (Maddison & Maddison, 1992) and COMPONENT (Page, 1993). However, tree mapping procedures based on the model of Goodman et al. (1979), which evaluate whether incongruence of gene and species trees could be due to sampling error (Page, 1990), were not performed because of the preliminary nature of this study.

For the cladistic analysis of the full length sequences, trees were rooted by designating *PHY* sequences from *Physcomitrella, Selaginella*, and *Adiantum capillus-veneris* L. (Okamoto et al., 1993) as the outgroups, because they are the only fully characterized *PHY* genes from nonangiosperms. For analysis of partial sequences in angiosperms, *Selaginella* was retained as an outgroup, along with the *PHY* sequences from the gymnosperms *Gingko* and *Pseudotsuga* that were determined during this analysis.

In all cladistic analyses, first, second, and third codon positions were equally weighted for the following reasons. First, empirically determined transition/transversion ratios did not vary significantly from 1.0 for any comparisons except for between closely related legume sequences that were differentiated by very few total substitutions (e.g., $\leq 3\%$ of all sites were variable). Second, results from cladistic analyses under certain differential weighting schemes are apparently the same as those from analyses under equal weighting schemes when taxonomic sampling is adequate (Albert et al., 1993; Cracraft & Helm-Bychowski, 1991). Finally, all codon positions may exhibit similar levels of homoplasy (see Chase et al., 1993); thus a rationale for excluding or differentially weighting codon positions is difficult to define. In these analyses, third codon positions, and perhaps many of the synonymous substitutions, were determined by bootstrap resampling analyses to be phylogenetically very informative, with confidence intervals for just the third codon position of between 90 and 100%, or at least as high as the values obtained for the first or second position.

Results

The orthology of fully sequenced *PHY* genes from various species to individual *PHY* loci from *Arabidopsis* has commonly been established by overall similarity (Dehesh et al., 1991; Heyer & Gatz, 1992a, b; Quail, 1991; Furuya, 1993). Similarities in gene expression and regulation have been used secondarily to imply orthology (Furuya,

1993). However, overall similarity may not reflect phylogeny, and phylogenetically related loci may differ in function due to mutations in *cis*-regulatory regions (e.g., Doyle, 1991; Li & Noll, 1994). Since orthology is best determined by shared ancestry, as evidenced by synapomorphies, cladistic analysis was used to determine the orthology of all available full length *PHY* sequences to those characterized from *Arabidopsis*. A single most parsimonious tree (Fig. 2) was generated in this analysis and it resolved the following monophyletic clades with strong (90–100%) bootstrap support: all monocot *PHYA*s, all dicot *PHYA*s, all *PHYA*s, all *PHYA*s + *Arabidopsis PHYC,* just *PHYB* and *PHYD* of *Arabidopsis,* just *PHYB*s and *Arabidopsis PHYD, Arabidopsis PHYE* + all *PHYB*s and *Arabidopsis PHYD,* all angiosperm *PHY*s, all angiosperm *PHY*s + *Psilotum,* and angiosperm *PHY*s + *Psilotum* + *Adiantum.* Seventy-eight trees were found by keeping all trees that were ≤30 steps longer than the most parsimonious one; all clades were retained in all trees that are 20 steps longer, except for *Arabidopsis PHYC* + all *PHYA*s. The two trees that were one step longer than the minimal length tree varied in their placement of *PHYC* as the sister group of either the *PHYA* or *PHYB/D/E* clade. These results thus suggest that, for example, the dicot and monocot *PHYA*s are orthologous, as are the dicot and rice *PHYB*s. Additionally, evidence is provided for the sister group relationship of *PHYE* with *PHYB* + *PHYD,* and for a later duplication giving rise to *Arabidopsis PHYB* or *PHYD.*

Using degenerate primers and amplification by PCR, target sequences from all five *Arabidopsis* genes, as well as from multiple *PHY* genes of other angiosperms, were recovered in single cloning experiments. Single *PHY* sequences were obtained from the nonangiosperms *Equisetum* and *Pseudotsuga* and two were obtained from *Gingko.* Inserts varied from 270 to 350 bp, and a region of insertion and deletion corresponding to residues 398 to 415 (App. 1) was eliminated from broad comparisons because nucleotide site homologies could not be determined. However, this region could be retained in narrower comparisons, where site homologies were more readily established, as in the Fabaceae data set (App. 4).

Similarly to the analysis of full-length sequences, angiosperm sequences determined in this study were cladistically analyzed to determine their orthology to the *PHY* loci of *Arabidopsis* (Figs. 3–5). Each sequence occurred in a monophyletic clade that included a single, specific *PHY* locus of *Arabidopsis,* providing evidence for distinct *PHY* subfamilies. Retention of a clade in a strict consensus tree (Figs. 2–5), resulting from the mhennig and branch-and-bound search options in Hennig86 or from heuristic options available in PAUP (see above), was considered good evidence of monophyly. Results from bootstrap resampling and decay analyses revealed that some clades were strongly supported (≥95%, d > 5–20).

The *Arabidopsis PHYA* sequence was included in a distinct monophyletic lineage in the dicot cladogram (Fig. 4). In the phylogenetic analysis of monocot sequences (Fig. 3), monocot orthologs of *PHYA* (Fig. 2) were substituted for *Arabidopsis PHYA.* Likewise, *Arabidopsis PHYA* was replaced by *Pisum PHYA* in the analysis of legume sequences (Fig. 5), also based on results depicted in Figure 2. A notable finding was that from three plant taxa, Ceratophyllaceae, Caryophyllaceae, and Fabaceae, two different PCR products were amplified that were determined to be most closely related to *Arabidopsis PHYA.* These are interpreted to be duplicated *PHYA* loci, and in legumes, the additional locus is here designated *PHYA′* (Fig. 5). These additional *PHYA*-related sequences appear to have arisen independently in the three plant groups (Figs. 4, 5). For example, the legume phytochrome phylogeny (Fig. 5) depicts this monophyletic *PHYA′* clade as being derived from within the legume *PHYA* lineage (which is thus paraphyletic). Also, it is well supported by a bootstrap value of 95%, and, in a global analysis of legume *PHYA′* with all other angiosperm loci, it is most closely related to legume *PHYA* (cladogram not shown). It thus appears that the evolution of the phytochrome gene family in the Fabaceae has involved the duplication of the *PHYA* locus. A similar argument can be made for the duplicated *PHYA* genes in Ceratophyllaceae and Caryophyllaceae (Fig. 4). In the *PHYA* subfamily, and in other cases described below, this pattern of diversification is attributed to the evolution of a new locus rather than to allelic diversity. With the exception of genes that are under frequency-dependent selection, such as alleles of the S-locus (Ioerger et al., 1990) and MHC-loci (Klein et al., 1993), levels of divergence among alleles at most loci are much lower (e.g., Gaut & Clegg, 1993; Thomas et al., 1993) than those observed among *PHYA* and the duplicated *PHYA* loci.

Sequences homologous to *Arabidopsis PHYC* were amplified commonly in monocots (Fig. 3). In dicots, only DNA of *Dianthus* yielded a sequence homologous to *Arabidopsis PHYC.* The homologs of *PHYC* in monocots were identified by their close relationship with just *Arabidopsis PHYC* in a global

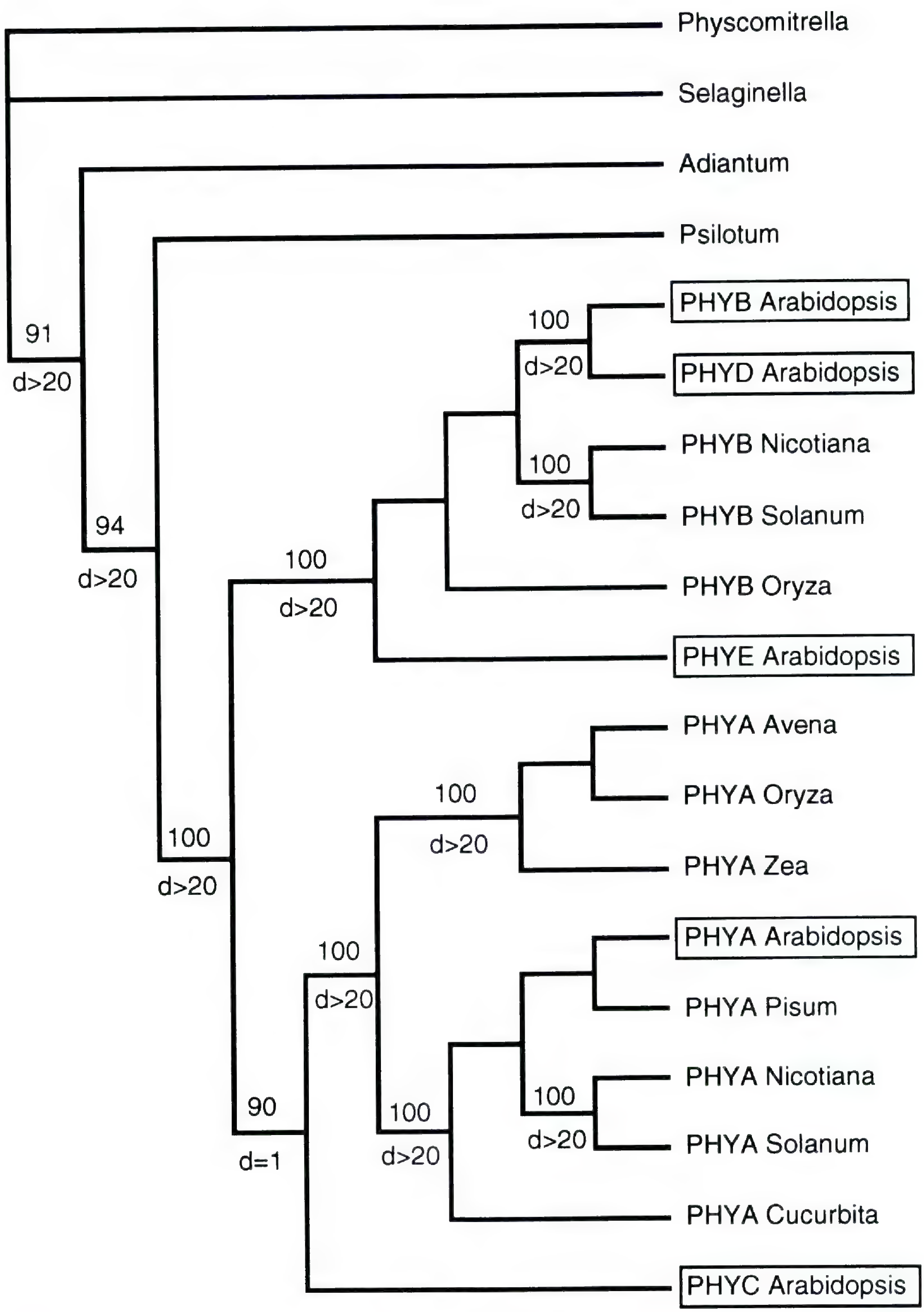

FIGURE 2. Single most parsimonious tree from analysis of 2637 variable nucleotide sites from the full-length phytochrome sequences. The length is 11,376, the CI = 0.459, and the RI = 0.502. Bootstrap values (from 500 replications) and decay indices are included on the best supported clades. The *Nicotiana* sequences were obtained from GenBank accessions X66784 and L10114.

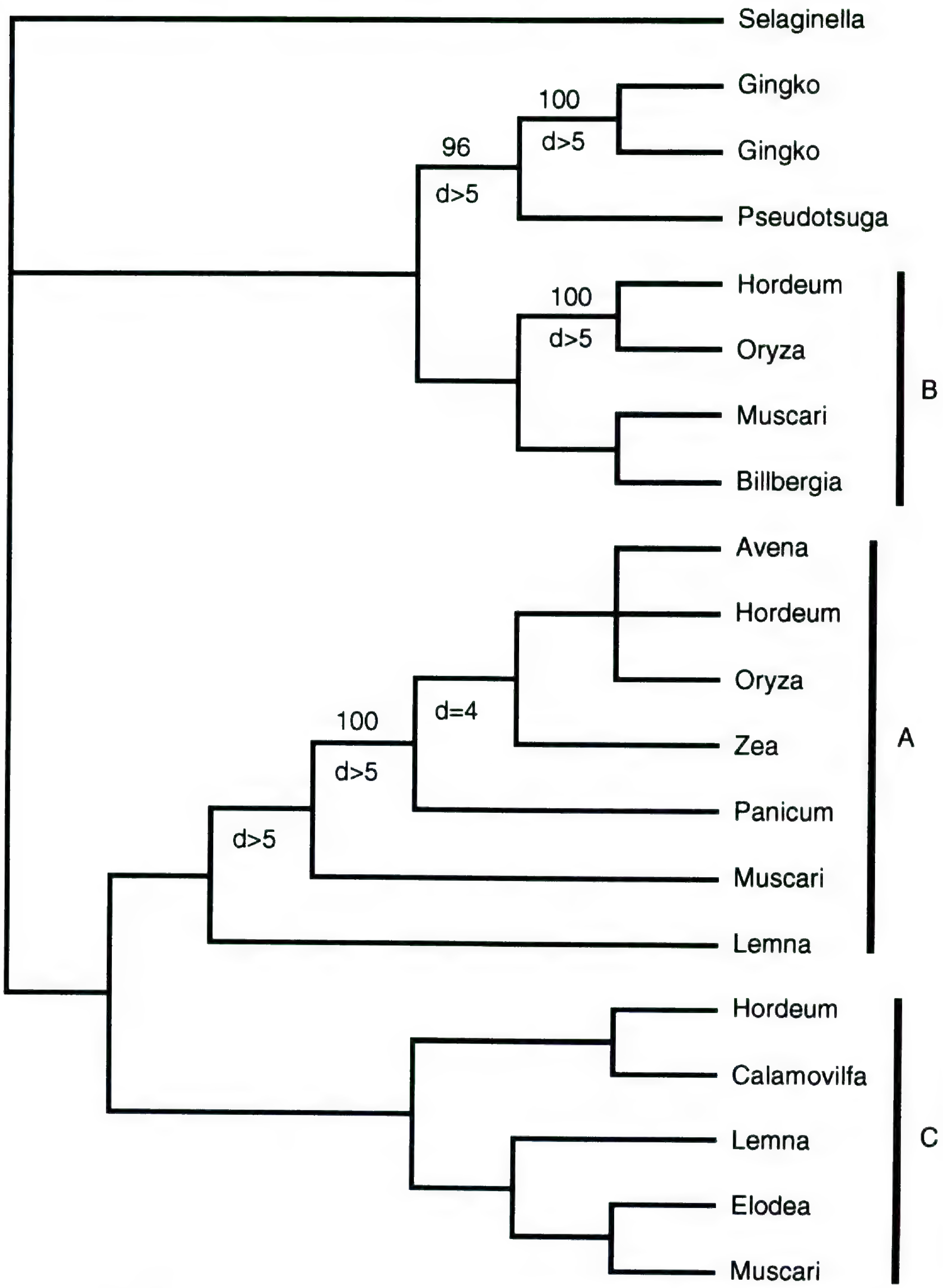

FIGURE 3. Single most parsimonious tree from analysis of all monocot sequence data, which comprised 169 informative sites. The length is 799, the CI = 0.44, and the RI = 0.52. Bootstrap values (from 500 replications) and decay indices are included on the best supported clades. Single uppercase letters to the right of the generic names are the names of the homologous *Arabidopsis PHY* loci.

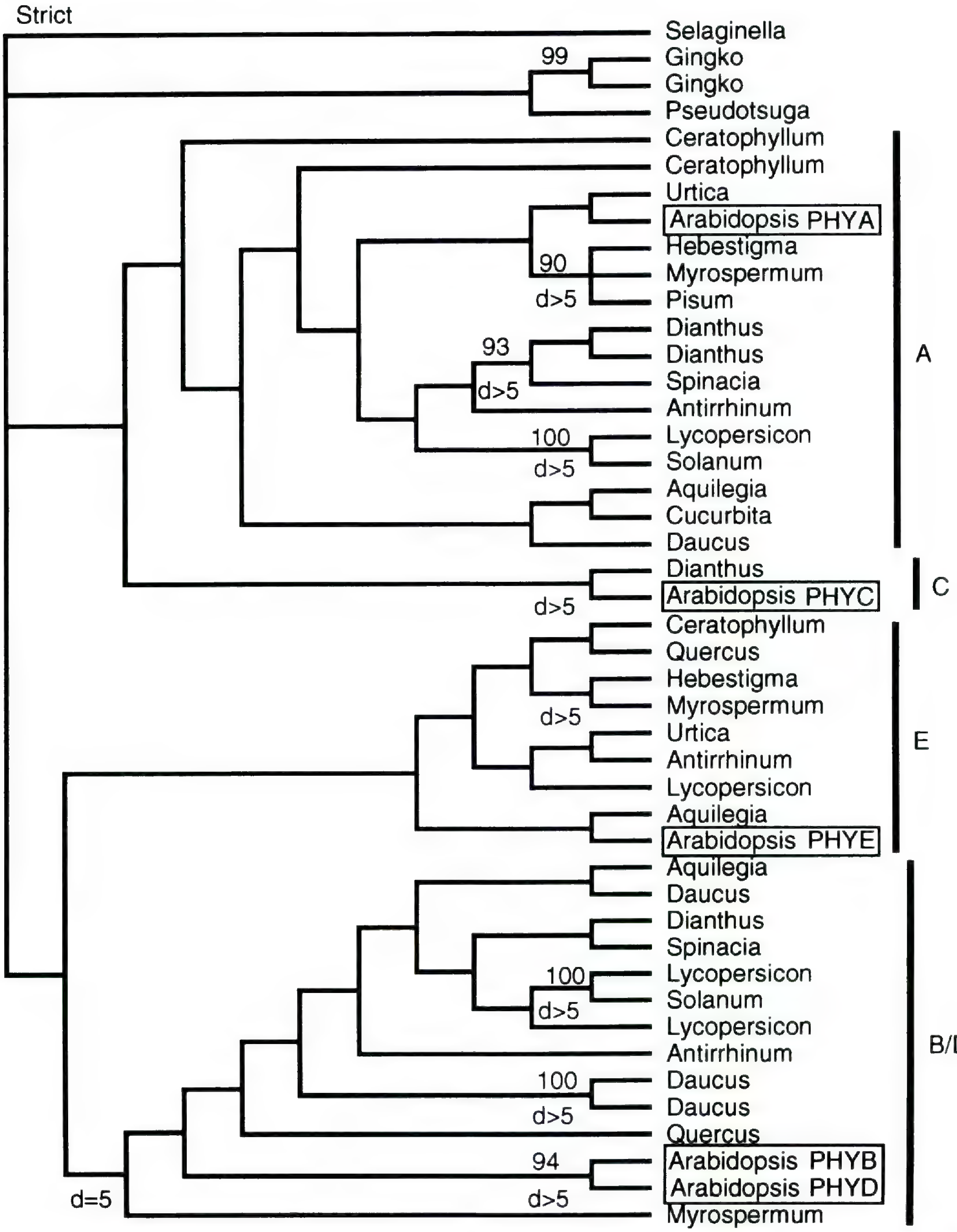

FIGURE 4. Strict consensus of four most parsimonious trees from analysis of all dicot sequence data, which comprised 172 informative sites. The length is 1743, the CI = 0.23, and the RI = 0.49. Bootstrap values (from 500 replications) and decay indices are included on the best supported clades. Single uppercase letters to the right of the generic names are the names of the homologous *Arabidopsis PHY* loci.

analysis (cladogram not shown). The *PHYC* homolog in *Dianthus* was identified by its sister group relationship with *Arabidopsis PHYC* (Fig. 4).

Sequences homologous to *Arabidopsis PHYE* were not amplified in monocots using the primer set described above. However, such homologs were commonly amplified in dicots, and the homology of these sequences to *PHYE* was readily established by the inclusion of *Arabidopsis PHYE* in monophyletic gene lineages (e.g., Fig. 4). Although the *Arabidopsis PHYE* sequence was not included in the legume data set (Fig. 5), two representative

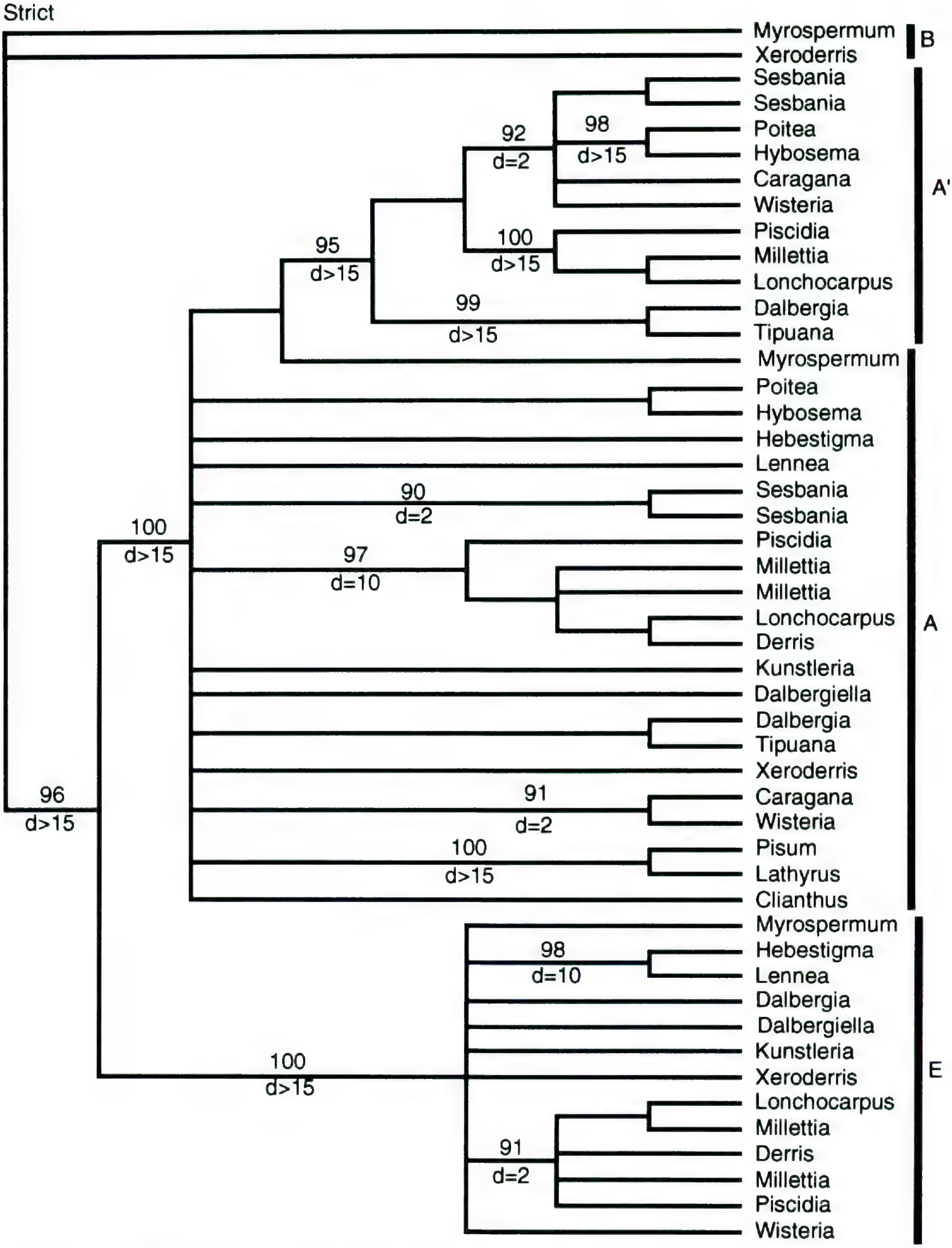

FIGURE 5. Strict consensus of 6500 minimal length trees generated from an mhennig* and branch and bound* search option on the 174 informative sites of the Fabaceae data set. Length = 545, CI = 0.534, and RI = 0.841. Bootstrap values (from 1000 replications) and decay indices are included on the best supported clades. Single uppercase letters to the right of the generic names represent the orthologs of the *Arabidopsis PHY* loci.

legumes were included in the dicot analysis shown in Figure 4, and these were part of the monophyletic gene lineage that included *Arabidopsis PHYE*. In the legume gene phylogeny, the bootstrap value for the *PHYE* clade was 100%, thus revealing how strongly this lineage is supported by the data in narrow comparisons at the taxonomic level of the family.

The evolution of genes related to *Arabidopsis PHYB* has been more complex, with the apparently independent duplication and divergence of *PHYB*-related genes in some dicot lineages, but perhaps not in monocot lineages (Figs. 3, 4). The notable pattern here is that the *Arabidopsis PHYB* and *PHYD* sequences are sister groups in comparisons including dicots (Figs. 2, 4), and together with the sequence from *Myrospermum* are the sister group of the other *PHYB/PHYD*-related sequences. Note that two *PHYB/D*-related sequences occur in *Lycopersicon*, forming a monophyletic clade, with a *PHYB*-related sequence from *Solanum*, that is separate from the clade containing *Arabidopsis PHYB* and *PHYD;* two of the *PHYB/D*-related sequences from *Daucus* also form a monophyletic clade (Fig. 4). This pattern could result from nonhomologous recombination between loci, but the hypothesis of recent divergence is consistent with the putative absence of additional *PHYB*-like sequences from monocots. Additionally, *PHYD* in *Arabidopsis* is apparently functionally distinct, as evidenced by its failure to compensate for the loss of *PHYB* function in *phyB* null mutants of *Arabidopsis* (Reed et al., 1993; Wester et al., 1994).

In the two trees with dicots (e.g., Figs. 2, 4), *PHYE* is the sister group to the *PHYB/PHYD* clade. Since *PHYD* and *PHYE* have not been amplified from monocots, the diversification of this part of the phytochrome gene family may have taken place only during the diversification of dicots. Further sampling from Nymphaeales, Piperales, Winterales, Laurales, and Magnoliales should address the question of whether the presence of just *PHYA*, *PHYB*, and *PHYC* is the ancestral condition in angiosperms. Notably, however, preliminary analysis of three sequences from *Piper* recently submitted to GenBank (Kolukisaoglu et al., unpublished), derived using a different primer pair, suggests that they are orthologs of *Arabidopsis PHYA*, *PHYB*, and *PHYC*. Alternatively, the inability to amplify *PHYD* and *PHYE* from monocots (and *Piper*) could mean that the oligonucleotide primers designed in recent studies do not recognize and amplify all *PHYD* and *PHYE* homologs. This alternative explanation should be evaluated in subsequent studies of the phytochrome gene family in monocots and in magnoliids with uniaperturate pollen.

The relationships among the angiosperm and nonangiosperm *PHY* lineages were evaluated in two additional types of analysis: (1) parsimony analyses of nucleotide sites homologous to the PCR target fragment, including all angiosperm *PHYA–E* paralogs and all nonangiosperm *PHY* sequences for which there were corresponding nucleotide data (about 330 bp); and (2) parsimony and distance analyses of amino acid sites homologous to the *Mougeotia* fragment (App. 2, about 300 amino acids), including from angiosperms shown in Appendix 1, and from gymnosperms determined in this study (with sites coded as missing). Patterns that emerged from the nucleotide sequence analyses included: (1) *Ceratodon*, *Physcomitrella*, *Selaginella*, *Equisetum*, *Gingko*, and *Pseudotsuga* most commonly occurred as sister groups of a *PHYB/D/E* clade; (2) *Mougeotia*, when not designated as the outgroup, was the sister lineage of the *PHYC* clade; (3) *PHYC*s were basal and paraphyletic in many cladograms rooted at *Mougeotia*; in others, or if *Mougeotia* was removed from analyses, a *PHYB/D* + *PHYE* clade and a *PHYA* + *PHYC* clade were most often resolved. Results of the amino acid sequence analyses indicate a major split between *PHYA* + *PHYC* and the *PHYB/D/E* clade, each with a set of nonangiosperms as sister group. The only common element of the two sets of analyses was the close relationship between the sequences available from *Gingko* and *Pseudotsuga* and the *PHYB/D/E* clade. Further, the robustness to perturbation of the data, which is found in the analysis of the full-length sequence data set (Fig. 2), is lost in these broad comparisons when the number of sites is limited.

Recently, single phytochrome sequence fragments (561–654 bp) from a number of nonangiosperms, including *Gnetum* and *Ephedra*, were deposited in GenBank (Kolukisaoglu et al., unpublished), bringing the total number of nonangiosperm homologous *PHY* sequence fragments available for nucleotide analysis to 15. Preliminary analyses of these sequences indicate that the data are still too fragmentary to draw conclusions regarding evolution of specific loci, especially as some of the nonangiosperm taxa represented by a single sequence are likely to have more than one *PHY* gene. Furthermore, organismal relationships depicted in these cladograms and neighbor-joining trees, except for the pairs *Ceratodon* + *Funaria* (both mosses) and *Metasequoia* + *Picea* (both conifers), are not well supported in bootstrap analysis.

Discussion

PHYTOCHROME EVOLUTION

The evolutionary pattern that emerges from phytochrome gene studies is that *PHY* gene diversity appears to be limited in nonangiosperms, where often a single gene is found, while diversity is much greater in angiosperms, where orthologs of the *PHYA*, *PHYB*, *PHYC*, *PHYD*, and *PHYE* genes discovered in *Arabidopsis* are present (Figs. 2–4). The data suggest that divergence of at least two, and most likely three, of the loci found in angiosperms preceded the diversification of flowering plants. For example, orthologs of *Arabidopsis PHYA*, *PHYB/D*, and *PHYC* have been detected in most angiosperm subclasses, and there is evidence for two loci in some nonangiosperm groups. Moreover, the model of a five member phytochrome gene family developed for *Arabidopsis* is probably not completely appropriate for all angiosperms. For example, though the PCR primers developed in this study annealed to and amplified dicot orthologs of the *Arabidopsis PHYA*, *PHYB/D*, *PHYC*, and *PHYE*, they annealed and amplified only three paralogs in monocots, *PHYA*, *PHYB/D*, and *PHYC*. The same primers applied to DNAs from the Fabaceae most commonly amplified *PHYA*, *PHYA'*, and *PHYE*; rarely did they amplify *PHYB/D* homologs, and they have yet to amplify *PHYC*. It is very possible that sequence divergence at the primer sites precludes the amplification of all loci present in some genomes, or that bias toward certain gene family members has occurred during amplification cycles; i.e., PCR selection or drift (sensu Wagner et al., 1994) has occurred. However, preliminary results indicate that the same loci are obtained from genera in Fabaceae when primers differing in GC content are used (Lavin, unpublished); likewise, certain variations of initial amplification conditions have not altered the set of loci detected in other angiosperms (Mathews, unpublished). Thus, it is likely that all five genes characterized from *Arabidopsis* did not precede the early diversification of angiosperms. Indeed, data presented here showing independent evolution of multiple *PHYB/D*-related sequences in *Arabidopsis*, *Lycopersicon*, and *Daucus* indicate that the divergence of the *PHYB* and *PHYD* loci in *Arabidopsis* occurred sometime well after the diversification of dilleniid families. Recent diversification of the phytochrome gene family in angiosperms is also suggested by the occurrence of *PHYA*-related sequences that have independently evolved in Ceratophyllaceae, Caryophyllaceae, and Fabaceae (see Fig. 5).

TEMPO OF SEQUENCE EVOLUTION

Using the 2-parameter model of Kimura (1981) to estimate distances among all pairs of full-length coding sequences, and a divergence time for *Selaginella* of 300 million years (Ma) ago (Townrow, 1968), the estimated overall rate of evolution of *PHY* lineages is 0.9 to 1.5 × 10^{-9} substitutions per site per year, or about ten times as fast as *rbc*L (Chase et al., 1993). In contrast, the rate of Jukes-Cantor corrected synonymous substitutions (K_S) among *PHY* sequences from pooid and panicoid grasses, with an estimated divergence time of 50 Ma (Doebley et al., 1990), and among tropical woody tribes of Fabaceae, with an estimated divergence time of 40 Ma (Herendeen, 1992; Wheeler & Baas, 1992) is four to five times as fast as *rbc*L (Zurawski et al., 1984; Doebley et al., 1990), or about 3.7 to 6.1 × 10^{-9} substitutions per site per year. Rates of Jukes-Cantor corrected nonsynonymous substitutions (K_A) estimated from pairwise comparisons with *Selaginella* for different portions of full-length phytochrome molecules (App. 1) indicate that the 594 bp including and proximal to the chromophore attachment site is the most conserved portion of the molecule (K_A = 3.2 to 4.6 × 10^{-10} subst./site/year), followed by the 2400 bp encoding the N-terminus (K_A = 4.0 to 5.4 × 10^{-10} subst./site/year), followed by 3384 bp comprising nearly the complete coding region (K_A = 4.3 to 6.2 × 10^{-10} subst./site/year). It is notable that K_S is consistently greater than K_A, even among the most closely related *PHY* loci (e.g., *Arabidopsis PHYB* and *PHYD*, and Fabaceae *PHYA* and *PHYA'*). The opposite pattern of substitution among codons associated with functional divergence has been used to suggest recent positive selection for divergent function among alleles (Nei & Hughes, 1991) and closely related loci (Ngai et al., 1993). However, the *PHY* loci might not be amenable to this comparison because of their more ancient divergence. Furthermore, the test cannot be precisely applied without more specific knowledge about codons associated with divergent functions.

In 42 relative rate tests (Wu & Li, 1985) used to evaluate the hypothesis that rates within and among the *PHY* loci are clocklike, 11 rate differences were significantly different ($P < 0.05$ or 0.01), given a model of rate constancy. All of these significant differences were among, rather than within, *PHY* lineages (Appendix 5), and are thus unlikely to be the source of spurious long-branch attractions in organismal phylogenies (Hendy & Penny, 1989).

IMPLICATIONS FOR ORGANISMAL PHYLOGENETIC ANALYSIS

Phytochrome sequence data is providing a high degree of phylogenetic resolution within the plant family Fabaceae, and this suggests that the phytochrome gene family, at the least, should be a promising source of data below the familial taxonomic level. Among other sorts of promising taxonomic characters is the presence of a novel legume locus related to *PHYA* (here referred to as *PHYA'*), which should eventually serve as a phylogenetic marker for a major subgroup of Fabaceae, or possibly among related families, once its taxonomic distribution becomes better known. One example of the significant phylogenetic implications that have been revealed so far is outlined below.

The phylogenetic relationships of the tropical woody papilionoid legume genera *Millettia, Lonchocarpus, Derris*, and putative close relatives of the tribe Millettieae remain poorly resolved (Polhill, 1994), despite recent comprehensive taxonomic studies (e.g., Evans et al., 1985; Geesink, 1981, 1984). *Millettia* is traditionally characterized only by its elastically dehiscent legume (Dunn, 1912), but the paraphyletic (and perhaps polyphyletic) nature of the genus has recently been confirmed by chloroplast DNA data (Liston, 1992). *Lonchocarpus* and *Derris* have indehiscent legumes; the former is traditionally distinguished by its wingless pods and a staminal tube with basal fenestrae, whereas *Derris* is traditionally characterized by winged pods and staminal tube lacking basal fenestrae (Geesink, 1981, 1984). However, these traditional characterizations have recently been disputed (Sousa & de Sousa, 1981; Sousa & Delgado, 1993). They argue that *Lonchocarpus* and *Derris* and relatives should be excluded from a close relationship with *Millettia* and allies, and placed closer to the genera of the tribe Dalbergieae, because of their indehiscent pods and putative indeterminate inflorescences. They also consider *Millettia* and close relatives to be part of the tribe Robinieae. In contrast, Polhill (1971, 1981) placed *Millettia, Lonchocarpus, Derris*, and close relatives together as a tribe separate from Dalbergieae and Robinieae (Polhill, 1981), because the three lineages have a similar phytochemistry and inflorescence structure (e.g., the pseudoracemose inflorescence).

Phytochrome sequence data from *PHYA*, *PHYA'*, and *PHYE* in these tropical woody papilionoid genera show much promise in providing at least some phylogenetic resolution to this group. The representatives of *Millettia, Lonchocarpus, Derris*, and certain allied genera (e.g., *Piscidia*) used in this analysis are consistently monophyletic in all minimal-length trees and in all three gene phylogenies (Fig. 5). Bootstrap confidence intervals above 90% in each individual gene phylogeny, and an amino acid deletion at position 405 (App. 4) in the *PHYA'* sequence, further support the monophyly of these genera. The phytochrome data suggest that this group is distinct from Dalbergieae (represented by *Dalbergia* and *Tipuana*), Robinieae (represented by *Sesbania, Hebestigma, Hybosema*, and *Lennea*), and certain other genera of Millettieae (e.g., *Kunstleria* and *Dalbergiella*). Such a grouping of *Millettia, Lonchocarpus, Derris*, and *Piscidia* (and presumably certain other genera when sampled) is consistent with chloroplast DNA data (Lavin, unpublished; see also Doyle & Doyle, 1993) and certain morphological data (Polhill, 1971). For example, this generic group is distinguished from other genera in the same tribe (such as *Kunstleria* and *Dalbergiella*), as well as the tribes Dalbergieae and Robinieae, by an inflorescence in which the flowers are fascicled along the raceme rachis, and by flowers in which the standard petals have claws that are abruptly contracted and subtended by calluses and inflexed auricles. This grouping is not consistent with whether the pods are dehiscent or not, or what type of nonprotein amino acid is accumulated in seed. That three different phytochrome loci, which are presumably under different evolutionary constraints, all reveal this same monophyletic group suggests that phytochrome sequence data will have a bearing on revealing those morphological characters that may best serve as phylogenetic markers in this taxonomically complex group of papilionoid legumes.

FUTURE DIRECTIONS

Phytochrome DNA sequence data, readily obtainable using PCR, are shown here to be informative regarding questions of organismal phylogeny in narrow comparisons, such as among closely related genera. However, the degree of resolution depicted in Figure 2 is promising for their use (if more nucleotide sites are included) in broader comparisons as well; notably, the branching order (except for the placement of *Psilotum*) is consistent with current hypotheses of plant phylogeny (summarized in Donoghue, 1994). Further, equally promising is the potential to use composite trees inferred from pairs of phytochrome loci that diverged prior to the diversification of angiosperms to determine evolutionary relationships among the

major angiosperm lineages in the manner Iwabe et al. (1989) inferred relationships among archaebacteria, eubacteria, and eukaryotes.

The data presented also raise intriguing questions concerning the evolution of individual phytochrome loci. For example, do monocots and a certain subgroup of magnoliids with uniaperturate pollen have only *PHYA*, *PHYB*, and *PHYC*, whereas in eudicots and another subgroup of magnoliids, diversification of the phytochrome gene family is much greater? If so, the *Arabidopsis* model is not completely applicable to monocots. As with the *PHYA'* locus in Fabaceae, the taxonomic distribution of *PHY* genes in monocots should provide phylogenetic insight into the divergence of monocots from dicots. Additionally, further phytochrome data, especially from nonangiosperms, potentially will reveal the history of phytochrome gene duplication events in the context of green plant phylogeny.

Exploration of such questions may be facilitated by a variety of tools; for example, preliminary data indicate that development of locus-specific PCR primers will be productive. So far, exclusively *PHYB*-related sequences have been determined from *Arabidopsis*, *Daucus*, *Quercus*, and *Spinacia* using a 3' *PHYB/D/E*-specific primer in combination with the conserved 5' primer.

Sequences determined in this study from taxa other than Fabaceae are available from GenBank under accession numbers UO8142–8184.

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Note added in proof.

Recent characterization of a genomic DNA clone from *Arabidopsis* containing *PHYC* indicates that this locus lacks the third intron shared by most of the other fully characterized *PHY* genes.

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```
                                10         20         30         40         50
                                 *          *          *          *          *
Selaginella (sm)[1]          ---------- ---------- ---------- ---------- MSTTKLTYSS
Ceratodon (cp)[2]            ---------- ---------- ---------- ---------- MSATKKTYSS
Adiantum (ac)[3]             ---------- ---------- ---------- ---------- MSSTRHSYSS
Arabidopsis PHYA (atA)[4]    ---------- ---------- ---------- -------MSG SRPT--QSSE
Cucurbita PHYA (cpA)[5]      ---------- ---------- ---------- -------MST SRPS--QSSS
Pisum PHYA (psA)[6]          ---------- ---------- ---------- -------MST TRPS--QSSN
Solanum PHYA (stA)[7]        ---------- ---------- ----MSTSLF ASDSDQLMSS SRPS--QSST
Avena PHYA (asA)[8]          ---------- ---------- ---------- -------MSS SRPA--SSSS
Oryza PHYA (osA)[9]          ---------- ---------- ---------- -------MSS SRPTQCSSSS
Zea PHYA (zmA)[10]           ---------- ---------- ---------- -------MSS SRPAHSSSSS
Arabidopsis PHYB (atB)[4]    --MVSGVGGS GGGRGGGRGG EEEPSSSHTP NNRRGGEQAQ SSGTKSLRPR
Arabidopsis PHYD (atD)[11]   MVSGGGSKTS GGEAASSGHR RSRHTSAAEQ AQSSANKALR WQNQQPQNHG
Arabidopsis PHYE (atE)[11]   ---------- ---------- ---------- ---------- MGFESSSSAA
Solanum PHYB (stB)[12]       ---------- ---------- -------MAS GSRTKHSHHS SSQAQSSGTS
Oryza PHYB (osB)[13]         MASGSRATPT RSPSSARPAA PRHQHHHSQS SGGSTSRAGG GGGGGGGGGG
Arabidopsis PHYC (atC)[4]    ---------- ---------- ---------- ---------- --MSSNTSRS
ANG                          ---------- ---------- ---------- ---------- ----------
CON                          ---------- ---------- ---------- ---------- ----------

             60         70         80         90        100        110        120
              *          *          *          *          *          *          *
sm   GSSAKSKHSV RVAQTTADAK LHAVYEESGE SGDSFDYSKS INATKSTGET IPAQ----AV -TAYLQRMQR
cp   TTSAKSKHSV RVAQTTADAA LEAVYEMSGD SGDSFDYSKS VGQSAE--SV P-----AGAV -TAYLQRMQR
ac   GGSGKSKHGR RIAQTSANAK LYAAYEESSE SGS-FDYSQS VSAGKEGI-- -----SSQLV -TAYLQRMQR
atA  GSRRSRHSAR IIAQTTVDAK LHADFE---E SGSSFDYSTS VRVTGPVV-- ENQPPRSDKV TTTYLHHIQK
cpA  NSGRSRHSTR IIAQTSVDAN VQADFE---E SGNSFDYSSS VRVTSDVS-- GDQQPRSDKV TTAYLHHIQK
psA  NSGRSRNSAR IIAQTTVDAK LHATFE---E SGSSFDYSSS VRVSGSVD-- GDQQPRSNKV TTAYLNHIQR
stA  TSSRSKHSAR IIAQTSIDAK LHADFE---E SGDSFDYSSS VRVTNVAE-- GEQRPKSDKV TTAYLHQIQK
asA  SRNRQSSQAR VLAQTTLDAE LNAEYE---E SGDSFDYSKL VEAQRDGP-- PVQQGRSEKV -IAYLQHIQK
osA  SRTRWSSRAR ILAQTTLDAE LNAEYE---E YGDSFDYSKL VEAQRTTG-- PEQQARSEKV -IAYLHHIQR
zmA  SRTRQSSRAR ILAQTTLDAE LNAEYE---E SGDSFDYSKL VEAQRSTP-- PEQQGRSGKV -IAYLQHIQR
atB  SNTESMSKSK AIQQYTVDAR LHAVFEQSGE SGKSFDYSQS LKTTTYGSSV PEQQ------ ITAYLSRIQR
atD  GGTESTNKNK AIQQYTVDAR LHAVFEQSGE SGKSFDYSQS LKTAPYDSSV PEQQ------ ITAYLSRIQR
atE  SNMKPQPQKS NTAQYSVDAA LFADFAQSIY TGKSFNYSKS VISPPN--HV PDEH------ ITAYLSNIQR
stB  NVNYKDSISK AIAQYTADAR LHAVFEQSGE SGKFFDYSQS VKTTTQ--SV PERQ------ ITAYLTKIQR
osB  GAAAAESVSK AVAQYTLDAR LHAVFEQSGA SGRSFDYTQS LRASPT--PS SEQQ------ IAAYLSRIQR
atC  CSTRSRQNSR VSSQVLVDAK LHGNFE---E SERLFDYSAS INLNM---PS SSCEIPSSAV -STYLQKIQR
ANG  ---------- ---Q---DA- ---------- ----F-Y--- ---------- ---------- ---YL--IQ-
CON  ---------- ---Q----A- ---------- ----F-Y--- ---------- ---------- ---YL---Q-

            130        140        150        160        170        180        190
              *          *          *          *          *          *          *
sm   GGLVQPFGCM LAV-EEGSFR VIAFSDNAGE MLDLMP-QSV PSL-GSGQQD VLTIGTDART LFTAAAS-AL
cp   EGLIQNFGCM VAV-EEPNFC VIAYSENASE FLDLIP-QAV PSM-GEM--D VLGIGTDIRT LFTPSSSAAL
ac   GGLVQQFGCL IAV-EEETFR VLHMCE-APE MLDVAT-QAV PTM-GQY--S RLCIGADVRT LLSPASASAL
atA  GKLIQPFGCL LAL-DEKTFK VIAYSENASE LLTMAS-HAV PSV-GEH--P VLGIGTDIRS LFTAPSASAL
cpA  GKLIQPFGCL LAL-DDKTFK VIAYSENAPE MLTMVS-HAV PSM-GDY--P VLGIGTDVRT IFTAPSASAL
psA  GKQIQPFGCL LAL-DEKTCK VVAYSENAPE MLTMVS-HAV PSV-GDH--P ALGIGTDIRT VFTAPSASAL
stA  GKFIQPFGCL LAL-DEKTLK VIAFSENAPE MLTMVS-HAV PSV-GEH--P VLGIGIDIRT IFTGPSGAAL
asA  GKLIQTFGCL LAL-DEKSFN VIAFSENAPE MLTTVS-HAV PSVD---DPP RLGIGTNVRS LFSDQGATAL
osA  AKLIQPFGCL LAL-DEKTFN VIALSENAPE MLTTVS-HAV PSVD---DPP KLRIGTNVRS LFTDPGTTAL
zmA  GKLIQPFGCL LAL-DEKSFR VIAFSENAPE MLTTVS-HAV PNVD---DPP KLGIGTNVRS LFTDPGATAL
atB  GGYIQPFGCM IAV-DESSFR IIGYSENARE MLGIMP-QSV PTLE---KPE ILAMGTDVRS LFTSSSSILL
atD  GGYTQPFGCL IAV-EESTFT IIGYSENARE MLGLMS-QSV PSIE-D-KSE VLTIGTDLRS LFKSSSYLLL
atE  GGLVQPFGCL IAV-EEPSFR ILGLSDNSSD FLGLLSLPST SHS-GEFDKV KGLIGIDART LFTPSSGASL
stB  GGHIQPFGCM IAV-DEASFR VIAYSENACE MLSLTP-QSV PSLE---KCE ILTIGTDVRT LFTPSSSVLL
osB  GGHIQPFGCT LAVADDSSFR LLAYSENTAD LLDLSPHHSV PSLDSSAVPP PVSLGADARL LFAPSSAVLL
atC  GMLIQPFGCL IVV-DEKNLK VIAFSENTQE MLGLIP-HTV PSME---QRE ALTIGTDVKS LFLSPGCSAL
ANG  ----Q-FGC- ---------- ----S-N--- -L-------- ---------- ----G----- -F-------L
CON  ----Q-FGC- ---------- ---------- -L-------- ---------- ----G----- ---------L
```

APPENDIX 1. All available full-length phytochrome amino acid sequences and 776 residues from *Ceratodon*. [1]Hanelt et al. (1992); [2]Thümmler et al. (1992); [3]Okamoto et al. (1993); [4]Sharrock & Quail (1989); [5]Sharrock et al. (1986); [6]Sato (1988); [7]Heyer & Gatz (1992a); [8]Hershey et al. (1985); [9]Kay et al. (1989); [10]Christensen & Quail (1989); [11]Clack et al. (1994); [12]Heyer & Gatz (1992b); [13]Dehesh et al. (1991). The triangle denotes the chromophore attachment site. The sequences amplified in this study correspond to residues 329–431.

```
              200        210        220        230        240        250        260
                *          *          *          *          *          *          *
sm   EKAAGAVDLS MLNPIWVQSK TSAKPFYAIV HRIDVGLVMD LEPVKASDTR VGSAAGALQS HKLAAKAISR
cp   EKAAATQDIS LLNPITVHCR RSGKPLYAIA HRIDIGIVID FEAVKMIDVP VSAAAGALQS HKLAARAITR
ac   DRVIGVVDVS MFNPITVQSR SSGKPFYAIL HRNDVGLVID LEPIRPDDAS I-TG-GALQS HKLAAKAIAR
atA  QKALGFGDVS LLNPILVHCR TSAKPFYAII HRVTGSIIID FEPVKPYEVP M-TAAGALQS YKLAAKAITR
cpA  LKALGFGEVT LLNPILVHCK TSGKPFYAIV HRVTGSLIID FEPVKPYEGP V-TAAGALQS YKLAAKAITR
psA  QKALGFAEVS LLNPILVHCK TSGKPFYAII HRVTGSLIID FEPVKPYEVP M-TAAGALQS YKLAAKAITR
stA  QKALGFGEVS LLNPVLVHCK NSGKPFYAIV HRVTGSLIID FEPVKPYEVP M-TAAGALQS YKLAAKAITR
asA  HKALGFADVS LLNPILVQCK TSGKPFYAIV HRATGCLVVD FEPVKPTEFP A-TAAGALQS YKLAAKAISK
osA  QKALGFADVS LLNPILVQCK TSGKPFYAIV HRATGCLVVD FEPVKPTEFP A-TAAGALQS YKLAAKAISK
zmA  QKALGFADVS LLNPILVQCK TSGKPFYAIV HRATGCLVVD FEPVKPTEFP A-TAAGALQS YKLAAKAISK
atB  ERAFVAREIT LLNPVWIHSK NTGKPFYAIL HRIDVGVVID LEPAR-TEDP ALSIAGAVQS QKLAVRAISQ
atD  ERAFVAREIT LLNPIWIHSN NTGKPFYAIL HRVDVGILID LEPAR-TEDP ALSIAGAVQS QKLAVRAISH
atE  SKAASFTEIS LLNPVLVHSR TTQKPFYAIL HRIDAGIVMD LEPAK-SGDP ALTLAGAVQS QKLAVRAISR
stB  ERAFGAREIT LLNPIWIHSK NSGKPFYAIL HRVDVGIVID LEPAR-TEDP ALSIAGAVQS QKLRSEGLFL
osB  ERAFAAREIS LLNPLWIHSR VSSNPFYAIL HRIDVGVVID LEPAR-TEDP ALSIAGAVQS QKLVVRAISR
atC  EKAVDFGEIS ILNPITLHCR SSSKPFYAIL HRIEEGLVID LEPVSPDEVP V-TAAGALRS YKLAAKSISR
ANG  --A------- -LNP------ ----PFYAI- HR-------D -EP------- ----AGA--S -KL-------
CON  ---------- --NP------ ----P-YAI- HR-------D -E-------- -----GA--S -KL-------

              270        280        290        300        310        320        330
                *          *          *          *          *          *          *
sm   LQSLP-GGDI GLLCDTVVEE VRDVTGYDLV MAYKFHEDEH GEVVAEIRRS DLEPYLGLHY PATDIPQASR
cp   LQALP-GGDI ELLCDTIVEE VRELTGYDRV MAFKFHEDEH GEVVAEIRRM DLEPYMGLHY PATDIPQASR
ac   LQSLP-GGDI GLLCDSVVEE VHELTGFDRV MAYKFHEDEH GEVVAEIRRT DLEPYIGLHY PATDIPQAAR
atA  LQSLP-SGSM ERLCDTMVQE VFELTGYDRV MAYKFHEDDH GEVVSEVTKP GLEPYLGLHY PATDIPQAAR
cpA  LQSLP-SGSM ARLCDTMVQE VFELTGYDRV MAYKFHDDDH GEVISEVAKP GLQPYLGLHY PATDIPQAAR
psA  LQSLA-SGSM ERLCDTMVQE VFELTGYDRV MAYKFHEDDH GEVIAEIAKP GLEPYLGLHY PATDIPQAAR
stA  LQSLP-SGSM ERLCDTMVQE VFELTGYDRV MGYKFHDDDH GEVVSEITKP GLEPYLGLHY PATDIPQAAR
asA  IQSLP-GGSM EVLCNTVVKE VFDLTGYDRV MAYKFHEDDH GEVFSEITKP GLEPYLGLHY PATDIPQAAR
osA  IQSLP-GGSM EVLCNTVVKE LFDLTGYDRV MAYKFHEDDH GEVFAEITKP GLEPYLGLHY PATDIPQAAR
zmA  IQSLP-GGSM EALCNTVVKE VFDLTGYDRV MAYKFHEDEH GEVFAEITKP GIEPYIGLHY PATDIPQAAR
atB  LQALP-GGDI KLLCDTVVES VRDLTGYDRV MVYKFHEDEH GEVVAESKRD DLEPYIGLHY PATDIPQASR
atD  LQSLP-SGDI KLLCDTVVES VRDLTGYDRV MVYKFHEDEH GEVVAESKRN DLEPYIGLHY PATDIPQASR
atE  LQSLP-GGDI GALCDTVVED VQRLTGYDRV MVYQFHEDDH GEVVSEIRRS DLEPYLGLHY PATDIPQAAR
stB  ICNHFLVGTL KLLCDTVVES VRELTGYDRV MVYKFHEDEH GEVVAESKRS DLEPYIGLHY PATDIPQASR
osB  LQALP-GGDV KLLCDTVVEH VRELTGYDRV MVYRFHEDEH GEVVAESRRS NLEPYIGLHY PATDIPQASR
atC  LQALP-SGNM LLLCDALVKE VSELTGYDRV MVYKFHEDGH GEVIAECCRE DMEPYLGLHY SATDIPQASR
ANG  -------G-- --LC---V-- ---LTGYDRV M-Y-FH-D-H GEV--E---- ---PY-GLHY -ATDIPQA-R
CON  -------G-- --LC---V-- ----TG-D-V M---FH-D-H GEV--E---- ---PY-GLHY -ATDIPQA-R
                                                                     >TARGET
              340        350        360        370        380        390        400
                *          *          *          *       ▼  *          *          *
sm   FLFMKNRVRM ICDCSAPPVK ITQDKELRQP ISLAGSTLRA PHGCHAQYMG NMGSVASLVM AMIINDNDE-
cp   FLLMKNRVRL IADCYASPVK LIQDPDIRQP VSLAGSTLRA PHGCHAQYMG NMGSIASLVM AVIINDNEE-
ac   FLFMKNRVRM ICDCRLPPVK LIQDKTLSQP MSLTGSTLRA PHSCHLQYMA NMDSIASLVM AVVVNEEDGE
atA  FLFMKNKVRM IVDCNAKHAR VLQDEKLSFD LTLCGSTLRA PHSCHLQYMA NMDSIASLVM AVVVNEEDGE
cpA  FLFMKNKVRM IVDCRAKHLK VLQDEKLQFD LTLCGSTLRA PHSCHLQYME NMNSIASLVM AVVVNEGDEE
psA  FLFMKNKVRM IVDCNAKHVK VLQDEKLPFD LTLCGSTLRA PHSCHLQYMA NMDSIASLVM AVVVNDSDED
stA  FLFMKNKVRM ICDCRAKHVK VVQDEKLPFD LTLCGSTLRA PHYCHLQYME NMNSIASLVM AVVVNDGDEE
asA  LLFMKNKVRM ICDCRARSIK VIEAEALPFD ISLCGSALRA PHSCHLQYME NMNSIASLVM AVVVNENEED
osA  FLFMKNKVRM ICDCRARSIK IIEDESLHLD ISLCGSTLRA PHSCHLQYME NMNSIASLVM AVVVNENEDD
zmA  FLFMKNKVRM ICDCRARSVK IIEDEALSID ISLCGSTLRA PHSCHLKYME NMNSIASLVM AVVVNENEED
atB  FLFKQNRVRM IVDCNATPVL VVQDDRLTQS MCLVGSTLRA PHGCHSQYMA NMGSIASLAM AVIINGNEDD
atD  FLFKQNRVRM IVDCYASPVR VVQDDRLTQF ICLVGSTLRA PHGCHAQYMT NMGSIASLAM AVIINGNEED
atE  FLFKQNRVRM ICDCNATPVK VVQSEELKRP LCLVNSTLRA PHGCHTQYMA NMGSVASLAL AIVVKGKD--
stB  FLFKQNRVRM IVDCHATPVR VTQDESLMQP LCLVGSTLRA PHGCHAQYMA NMGSIASLTL AVIINGNDEE
osB  FLFRQNRVRM IADCHAAPVR VIQDPALTQP LCLVGSTLRS PHGCHGQYMA NMGSIASLVM AVIISSGGDD
atC  FLFMRNKVRM ICDCSAVPVK VVQDKSLSQP ISLSGSTLRA PHGCHAQYMS NMGSVASLVM SVTINGSDSD
ANG  -LF--N-VRM I-DC-A---- ------L--- --L--S-LR- PH-CH--YM- NM-S-ASL-- ----------
CON  -L---N-VR- I-DC------ ---------- --L--S-LR- PH-CH--YM- NM-S--SL-- ----------
```

Appendix 1. Continued.

```
            410        420        430        440        450        460        470
              *          *          *          *          *          *          *
sm   -PSGGGGGGG QHKGRRLWGL VVCHHTSPRS VPF-LRSACE FLMQVFGLQL NMEAAVAAHV REKHILRTQT
cp   -----YSRGA IQRGRKLWGL VVCQHTSPRT VPFPLRSVCE FLMQVFGMQL NLHVELAAQL REKHILRTQT
ac   ------SAGH SSQGIKLWGL VVCHHTSPRY VPFPVRSACE FLMQVFSLQL NMEVGMAAQV REKHILRTQT
atA  GD-APDATTQ PQKRKRLWGL VVCHNTTPRF VPFPLRYACE FLAQVFAIHV NKEVELDNQM VEKNILRTQT
cpA  NE---GPALQ QQKRKRLWGL VVCHNSSPRF VPFPLRYACE FLAQVFAIHV NKELELENQI IEKNILRTQT
psA  GD--SADAVL PQKKKRLWGL VVCHNTTPRF VPFPLRYACE FLAQVFAIHV NKEIELEYQI LEKNILRTQT
stA  GE--SSDSSQ SQKRKRLWGL VVSHNTTPRF APFPLRYACE FLAQVFAILV NKELELENQF LEKNILRTQT
asA  DEAESEQPAQ QQKKKKLWGL LVCHHESPRY VPFPLRYACE FLAQVFAVHV NREFELEKQL REKNILKMQT
osA  DEVGADQPAQ QQKRKKLWGL LVCHHESPRY VPFPLRYACE FLAQVFAVHV NKEFELERQV REKSILRMQT
zmA  DEPEPEQPPQ QQKKKRLWGL IVCHHESPRY VPFPLRYACE FLAQVFAVHV NKEFELEKQI REKNILRMQT
atB  G----SNVAS GRSSMRLWGL VVCHHTSSRC IPFPLRYACE FLMQAFGLQL NMELQLALQM SEKRVLRTQT
atD  G---NGVNTG GRNSMRLWGL VVCHHTSARC IPFPLRYACE FFMQAFGLQL NMELQLASQV SEKRVLRMQT
atE  ---------- ---SSKLWGL VVGHHCSPRY VPFPLRYACE FLMQAFGLQL QMELQLASQL AEKKAMRTQT
stB  ------AVGG GRNSMRLWGL VVGHHTSVRS IPFPLRYACE FLMQAFGLQL NMELQLASQL SEKHVLRTQT
osB  D--HNIARGS IPSAMKLWGL VVCHHTSPRC IPFPLRYACE FLMQAFGLQL NMELQLAHQL SEKHILRTGT
atC  E-----MNRD LQTGRHLWGL VVCHHASPRF VPFPLRYACE FLTQVFGVQI NKEAESAVLL KEKRILQTQS
ANG  ---------- ------LWGL -V-H----R- -PFPLRYACE F--Q-F---- --E------- -EK-------
CON  ---------- ------LWGL -V------R- -PF--R--CE F--Q-F---- ---------- -EK-------
                                               <

            480        490        500        510        520        530        540
              *          *          *          *          *          *          *
sm   LLCDMLLRDA -PIGIVSQSP NIMDLVKCDG AALYYGKRFW LLGITPSEAQ IKDIAEWLLE HH-KDSTGLS
cp   LLCDMLMRDA -PLGIVSQTP NIMDLVKCDG AALYYGKRVW LLGTTPTENQ IKEIADWLLE HH-MDSTGLS
ac   LLCDMLLRDA -PIGIVSQSP NIMDLVTCDG AALYYGKKCW LLGTTPTEAQ IVDIAAWLLD CH-KDSTGLS
atA  LLCDMLMRDA -PLGIVSQSP NIMDLVKCDG AALLYKDKIW KLGTTPSEFH LQEIASWLCE YH-MDSTGLS
cpA  LLCDMLMRDA -PLGIVSRSP NIMDLVKSDG AALLYKKKIW RLGLTPNDFQ LLDIASWLSE YH-MDSTGLS
psA  LLCDMLMRDA -PLGIVSQSP NIMDLVKCDG AALFYRNKLW LLGATPTESQ LREIALWMSE YH-TDSTGLS
stA  LLCDMLMRDA -PLGIVSQSP NIMDLIKCDG AALLYKNKIH RLGMNPSDFQ LHDIVSWLCE YH-TDSTGLS
asA  MLSDMLFREA SPLTIVSGTP NIMDLVKCDG AALLYGGKVW RLQNAPTESQ IHDIAFWLSD VH-RDSTGLS
osA  MLSDMLFRES SPLSIVSGTP NIMDLVKCDG AALLYGGKVW RLQNAPTESQ IRDIAFWLSD VH-RDSTGLS
zmA  MLSDMLFKES SPLSIVSGSP NIMDLVKCDG AALLYGDKVW RLQTAPTESQ IRDIAFWLSE VH-GDSTGLS
atB  LLCDMLLRDS -PAGIVTQSP SIMDLVKCDG AAFLYHGKYY PLGVAPSEVQ IKDVVEWLLA NH-ADSTGLS
atD  LLCDMLLRDS -PAGIVTQRP SIMDLVKCNG AAFLYQGKYY PLGVTPTDSQ INDIVEWLVA NH-SDSTGLS
atE  LLCDMLLRDT -VSAIVTQSP GIMDLVKCDG AALYYKGKCW LVGVTPNESQ VKDLVNWLVE NHGDDSTGLT
stB  LLCDMLLRDS -PPGIVTQSP SIMDLVKCDG ALLYYQGKYY PLGVTPTEAQ IKDIVEWLLA YH-GDSTGLS
osB  LLCDMLLRDS -PTGIVTQSP SIMDLVKCDG AALYYHGKYY PLGVTPTEVQ IKDIIEWLTM CH-GDSTGLS
atC  VLCDMLFRNA -PIGIVTQSP NIMDLVKCDG AALYYRDNLW SLGVTPTETQ IRDLIDWVLK SH-GGNTGFT
ANG  -L-DML---- ----IV---P -IMDL-K--G A---Y----- -----P---- ------W--- -H----TG--
CON  -L-DML---- ----IV---P -IMDL----G A---Y----- -----P---- ------W--- -H----TG--

            550        560        570        580        590        600        610
              *          *          *          *          *          *          *
sm   TDSLADAGYP GAASLGDEVC GMAAAKITAK DFLFWFRSHT AKEVKWGGAK HDPDDKDDGR KMHPRSSSKA
cp   TDSLADANYP GAHLLGDAVC GMAAAKITAK DFLFWFRSHT ATEVKWGGAK HDPDEKDDGR KMHPRSSFKA
ac   TDSLAKTGYP EASCLGDAVC GLAAAKITAT DFLFWFRSHT AKEVRWGGAR HDPEERDDGR RMHPRSSFKA
atA  TDSLHDAGFP RALSLGDSVC GMAAVRISSK DMIFWFRSHT AGEVRWGGAK HDPDDRDDAR RMHPRSSFKA
cpA  TDSLYDAGYP GAIALGDEVC GMAAVRITNN DMIFWFRSHT ASEIRWGGAK HEHGQKDDAR KMHPRSSFKA
psA  TDSLSDAGFP GALSLSDTVC GMAAVRITSK DIVFWFRSHT AAEIRWGGAK HEPGDQDDGR KMHPRSSFKA
stA  TDSLYDAGFP GALALGDAVC GMAAVRISDK DWLFWYRSHT AAEVRWGGAK HEPGEKDDGR KMHPRSSFKG
asA  TDSLHDAGYP GAAALGDMIC GMAVAKINSK DILFWFRSHT AAEIRWGGAK NDPSDMDDSR RMHPRLSFKA
osA  TDSLHDAGYP GAAALGDMIC GMAVAKINSK DILFWFRSHT AAEIRWGGAK HDPSDKDDSR RMHPRLSFKA
zmA  TDSLQDAGYP GAASLGDMIC GMAVAKITSK DILFWFRSHT AAEIKWGGAK HDPSDKDDNR RMHPRLSFKA
atB  TDSLGDAGYP GAAALGDAVC GMAVAYITKR DFLFWFRSHT AKEIKWGGAK HHPEDKDDGQ RMHPRSSFQA
atD  TDSLGDAGYP RAAALGDAVC GMAVACITKR DFLFWFRSHT EKEIKWGGAK HHPEDKDDGQ RMNPRSSFQT
atE  TDSLVDAGYP GAISLGDAVC GVAAAEFSSK DYLLWFRSNT ASAIKWGGAK HHPKDKDDAG RMHPRSSFTA
stB  TDSLPDAGYP GAASLGDAVC GMAVAYITSK DFLFWFRSHT AKEIKWGGAK HHPEDKDDGQ RMHPRSSFKA
osB  TDSLADAGYS GAAALGDAVS GMAVAYITPS DYLFWFRSHT AKEIKWGGAK HHPEDKDDGQ RMHPRSSFKA
atC  TESLMESGYP DASVLGESIC GMAAVYISEK DFLFWFRSST AKQIKWGGAR HDPNDR-DGK RMHPRSSFKA
ANG  T-SL---G-- -A--L----- G-A------- D---W-RS-T -----WGGA- -------D-- -M-PR-SF--
CON  T-SL------ -A--L----- G-A------- D---W-RS-T -----WGGA- -------D-- -M-PR-S---
```

APPENDIX 1. Continued.

```
              620        630        640        650        660        670        680
                *          *          *          *          *          *          *
sm   FLEVVKRRSL PWEDVEMDAI HSLQLILRGS FQDIDDSDTK TM-IHAR--- LNDLKLQGMD ELSTVANEMV
cp   FLEVVNKRSP PWEDVEMDAI HSLQLILRGS FRDIADSDTK TM-IHAR--- LNDLKLQGVE ERNALANEMS
ac   FLEVVKQQSL PWEDVEMDAI HSLQLILRGS FQDIDDSNTK TM-IHAR--- LNDLKLQGLD ELSTVASEMV
atA  FLEVVKTRSL PWKDYEMDAI HSLQLILRNA FKDSETTDVN TKVIYSK--- LNDLKIDGIQ ELEAVTSEMV
cpA  FLEVVKTRSL PWKDYEMDAI HSLQLILRNT FKDTDATEIN RKSIQTT--- LGDLKIEGRQ ELESVTSEMV
psA  FLEVVKARSV PWKDFEMDAI HSLQLILRNA SKDTDIIDLN TKAINTR--- LNDLKIEGMQ ELEAVTSEMV
stA  FLEVVKTRSI PWKDYEMDRI HSLQLILRNA FKDADAVNSN TISIHTK--- LNDLKIDGMQ ELEAVTAEMV
asA  FLEVVKMKSL PWSDYEMDAI HSLQLILRGT LNDASKPKRE ASLDNQI--- -GDLKLDGLA ELQAVTSEMV
osA  FLEVVKMKSL PWNDYEMDAI HSLQLILRGT LNDDIKPTRA ASLDNQV--- -GDLKLDGLA ELQAVTSEMV
zmA  FLEVVKTKSL PWSDYEMDAI HSLQLILRGT LNDASKPAQA SGLDNQI--- -GDLKLDGLA ELQAVTSEMV
atB  FLEVVKSRSQ PWETAEMDAI HSLQLILRDS FKESEAAMNS KVVDGVVQPC RDMAGEQGID ELGAVAREMV
atD  FLEVVKSRCQ PWETAEMDAI HSLQLILRDS FKESEAMDSK AAAAGAVQPH GDDMVQQGMQ EIGAVAREMV
atE  FLEVAKSRSL PWEISEIDAI HSLRLIMRES FTSSRPVLSG NGVARDAN-- ---------- ELTSFVCEMV
stB  FLEVVKSRSS PWENAEMDAI HSLQLILRDS FKDAEASNSK AIVHAH---- LGEMELQGID ELSSVAREMV
osB  FLEVVKSRSL PWENAEMDAI HSLQLILRDS FRDSAEGTSN SKAIVNGQVQ LGELELRGID ELSSVAREMV
atC  FMEIVRWKSV PWDDMEMDAI NSLQLIIKGS LQEEH---SK TVVDVP---- LVDNRVQKVD ELCVIVNEMV
ANG  F-E------- PW---E-D-I -SL-LI---- ---------- ---------- ---------- E------EMV
CON  F-E------- PW---E-D-I -SL-LI---- ---------- ---------- ---------- E------EM-

              690        700        710        720        730        740  INTRON 750
                *          *          *          *          *          *    |     *
sm   RLIETATAPI LAVDSSGFIN GWNAKVADVT GLPVTEAMGR SLAKELVLHE SADMVERLLY LALQGDEEQN
cp   RVLETAAAPI LAVDSRGMIN AWNAKIAQVT GLPVEEAMHC SLTKDLVLDE SVVVVERLLS LALQGEEEQN
ac   RLIETATAPI LAVDGQGLIN GWNGKVAELT GLSFETAMGK SLAKELVHEE SKTIVERVLH LALEGEEEQD
atA  RLIETATVPI LAVDSDGLVN GWNTKIAELT GLSVDEAIGK HFLT-LVEDS SVEIVKRMLE NALEGTEEQN
cpA  RLIETATVPI LAVDLDGLIN GWNTKIAELT GLPVDKAIGK HLLT-LVEDS SVEVVRKMLF LALQGQEEQN
psA  RLIETATVPI LAVDVDGTVN GWNIKIAELT GLPVGEAIGK HLLT-LVEDS STDIVKKMLN LALQGEEEKN
stA  RLIETASVPI FAVDVDGQVN GWNTKVAELT GLPVDEAIGK HLLT-LVEDS SVDTVNKMLE LALQGQEERN
asA  RLMETATVPI LAVDGNGLVN GWNQKAAELT GLRVDDAIGR HILT-LVEDS SVPVVQRMLY LALQGKEEKE
osA  RLMETATVPI LAVDSNGLVN GWNQKVAELT GLRVDEAIGR HILT-VVEES SVPVVQRMLY LALQGKEEKE
zmA  RLMETATVPI LAVDGNGLVN GWNQKVAELS GLRVDEAIGR HILT-LVEDS SVSLVQRMLY LALQGREEKE
atB  RLIETATVPI FAVDAGGCIN GWNAKIAELT GLSVEEAMGK SLVSDLIYKE NEATVNKLLS RALRGDEEKN
atD  RLIETATVPI FAVDIDGCIN GWNAKIAELT GLSVEDAMGK SLVRELIYKE YKETVDRLLS CALKGDEGKN
atE  RVIETATAPI FGVDSSGCIN GWNKKTAEMT GLLASEAMGK SLADEIVQEE SRAALESLLC KALQGEEEKS
stB  RLIETATAPI FAVDVEGRIN GWNAKVAELT GVSVEEAMGK SLVHDLVYKE SQETAEKLLY NALRGEEDKN
osB  RLIETATVPI FAVDTDGCIN GWNAKVAELT GLSVEEAMGK SLVNDLIFKE SEETVNKLLS RALRGDEDKN
atC  RLIDTAAVPI FAVDASGVIN GWNSKAAEVT GLAVEQAIGK P-VSDLVEDD SVETVKNMLA LALEGSEERG
ANG  R---TA--PI --VD--G--N GWN-K-AE-- G-----A-G- ---------- --------L- -AL-G-E---
CON  R---TA--PI --VD--G--N -WN-K-A--- G-----A--- ---------- --------L- -AL-G-E---

              760        770        780        790        800        810        820
                *          *          *          *          *          *          *
sm   VELKLKTFGG QKDKEAVIL- --VVNACASR DVSDNVVGVC FVGQDVTGQK VVMDKFTRIQ GDYKAIVQNP
cp   VEIKLKTFGT QTTERAVIL- --IVNACCSR DASDFVVGVF FVGQDVTEQR MFMDRFTRIQ GGEKTTVQDP
ac   IEIHLRTYDQ HKQKGVVIL- --IVNTCCSR DVSNNVVGVC FVGQDVTGQK LVLDRFIRIQ GDYKAIVQSL
atA  VQFEIKTHLS RADAGPISL- --VVNACASR DLHENVVGVC FVAHDLTGQK TVMDKFTRIE GDYKAIIQNP
cpA  VQFEIKTHGS HIEVGSISL- --VVNACASR DLRENVVGVF FVAQDITGQK MVMDKFTRLE GDYKAIVQNP
psA  VQFEIKTHGD QVESGPISL- --IVNACASK DLRENVVGVC FVAQDITAQK TVMDKFTRIE GDYKAIVQNP
stA  VEFEIKTHGP SRDSSPISL- --IVNACASK DVRDSVVGVC FIAQDITGQK SIMDKFTRIE GDYRAIIQNP
asA  VRFEVKTHGP KRDDGPVIL- --VVNACASR DLHDHVVGVC FVAQDMTVHK LVMDKFTRVE GDYKAIIHNP
osA  VKFEVKTHGS KRDDGPVIL- --VVNACASR DLHDHVVGVC FVAQDMTVHK LVMDKFTRVE GDYKAIIHNP
zmA  VRFELKTHGS KRDDGPVIL- --VVNACASR DLHDHVVGVC FVAQDMTVHK LVMDKFTRVE GDYKAIIHNP
atB  VEVKLKTFSP ELQGKAVFV- --VVNACSSK DYLNNIVGVC FVGQDVTSQK IVMDKFINIQ GDYKAIVHSP
atD  VEVKLKTFGS ELQGKAMFV- --VVNACSSK DYLNNIVGVC FVGQDVTGHK IVMDKFINIQ GDYKAIIHSP
atE  VMLKLRKFGQ NNHPDYSSDV CVLVNSCTSR DYTENIIGVC FVGQDITSEK AITDRFIRLQ GDYKTIVQSL
stB  VEIKLRTFGA EQLEKAVFV- --VVNACA-R DYTNNIVGVC FVGQDVTGEK VVMDKFINIQ GDYKAIVHSP
osB  VEIKLKTFGP EQSKGPIFV- --IVNACSTR DYTKNIVGVC FVGQDVTGQK VVMDKFINIQ GDYKAIVHNP
atC  AEIRIRAFGP KRKSSPVEL- --VVNTCCSR DMTNNVLGVC FIGQDVTGQK TLTENYSRVK GDYARIMWSP
ANG  ---------- ---------- ---VN-C--- D------GV- F---D-T--K ---------- GDY--I----
CON  ---------- ---------- ---VN-C--- D------GV- F---D-T--- ---------- G---------
```

APPENDIX 1. Continued.

```
              830        840        850        860        870        880        890
                *          *          *          *          *          *          *
sm   NPLIPPIFGA DEFGYCSEWN PAMEKLSGWR REEVLGKMLV GEIFGIQMMY CRLKGQDAVT KFMIVLNSAA
cp   HPLMRPSFDG DEFGRTFKRN SALGGL---- ---------- ---------- ---------- ----------
ac   NPLIPPIFGA DEYGFCSEWN AAMEKLSNWR REEVLGKMLV GEIFGLQMVC CRLQGQDVVT KLMIVLNDAV
atA  NPLIPPIFGT DEFGWCTEWN PAMSKLTGLK REEVIDKMLL GEVFGTQKSC CRLKNQEAFV NLGIVLNNAV
cpA  NPLIPPIFGS DEFGWCSEWN PAMAKLTGWS REEVIDKMLL GEVFGVHKSC CRLKNQEAFV NLGIVLNNAM
psA  NQLIPPIFGT DEFGWCCEWN AAMIKLTGWK REEVMDKMLL GEVFGTQMSC CRLKNQEAFV NFGIVLNKAM
stA  HPLIPPIFGT DQFGWCSEWN SAMTMLTGWR RDDVMDKMLL GEVFGTQAAC CRLKNQEAFV NFGVILNNAI
asA  NPLIPPIFGA DEFGWCSEWN AAMTKLTGWN RDEVLDKMLL GEVFDSSNAS CPLKNRDAFV SLCVLINSAL
osA  SPLIPPIFGA DEFGWCSEWN AAMTKLTGWH RDEVINKMLL GEVFDSTNAS CLVKNKDAFV SLCILINSAL
zmA  NPLIPPIFGA DQFGWCSEWN AAMTKLTGWH RDEVVDKMLL GEVFNSSNAS CLLKSKDAFV RLCIVINSAL
atB  NPLIPPIFAA DENTCCLEWN MAMEKLTGWS RSEVIGKMIV GEVFG---SC CMLKGPDALT KFMIVLHNAI
atD  NPLIPPIFAA DENTCCLEWN TAMEKLTGWP RSEVIGKLLV REVFG---SY CRLKGPDALT KFMIVLHNAI
atE  NPLIPPIFAS DENACCSEWN AAMEKLTGWS KHEVIGKMLP GEVFG---VF CKVKCQDSLT KFLISLYQGI
stB  NPLIPPIFAS DENTCCSEWN TAMEKLTGWS RGEIVGKMLV GEIFG---SC CRLKGPDAMT KFMIVLHNAI
osB  NPLIPPIFAS DENTCCSEWN TAMEKLTGWS RGEVVGKLLV GEVFG---NC CRLKGPDALT KFMIVLHNAI
atC  STLIPPIFIT NENGVCSEWN NAMQKLSGIK REEVVNKILL GEVFTTDDYG CCLKDHDTLT KLRIGFNAVI
ANG  --LIPPIF-- -----C-EWN -AM-KL-G-- ------K--- -E-F------ C--K------ ----------
CON  --L--P-F-- ---------N -A---L---- ------K--- -E-F------ C--------- ----------

              900        910        920        930        940        950        960
                *          *          *          *          *          *          *
sm   DGQ-DTEKFP FAFFDRQGKY VEALLTATKR ADAEGSITGV FCFPHIASAE LQQALTVQRA TEKVALSKLK
cp   ---------- ---------- ---------- ---------- ---------- ---------- ----------
ac   NGQ-ESEKFP LVFYDRNGRR VEALLIASKR TDADGRITGV FCFLHTASPE LLQALIIKRA KEKV----DK
atA  TSQ-DPEKVS FAFFTRGGKY VECLLCVSKK LDREGVVTGV FCFLQLASHE LQQALHVQRL AERTAVKRLK
cpA  CGQ-DPEKAS FGFLARNGMY VECLLCVNKI LDKDGAVTGF FCFLQLPSHE LQQALNIQRL CEQTALKRLR
psA  TGL-ETEKVP FGFFSRKGKY VECLLSVSKK IDAEGLVTGV FCFLQLASPE LQQALHIQRL SEQTALKRLK
stA  TGQ-ESEKIP FGFFARYGKY VECLLCVSKR LDKEGAVTGL FCFLQLASHE LQQALHVQRL SEQTALKRLK
asA  AGE-ETEKAP FGFFDRSGKY IECLLSANRK ENEGGLITGV FCFIHVASHE LQHALQVQQA SEQTSLKRLK
osA  AGD-ETEKAP FSFFDRNGKY IECLLSVNRK VNADGVITGV FCFIQVPSHE LQHALHVQQA SQQNALTKLK
zmA  AGE-EAEKAS FGFFDRNEKY VECLLSVNRK VNADGVVTGV FCFIHVPSDD LQHALHVQQA SEQTAQRKLK
atB  GGQ-DTDKFP FPFFDRNGKF VQALLTANKR VSLEGKVIGA FCFLQIPSPE LQQALAVQRR QDTECFTKAK
atD  GGQ-DTDKFP FPFFDRKGEF IQALLTLNKR VSIDGKIIGA FCFLQIPSPE LQQALEVQRR QESEYFSRRK
atE  AGDNVPESSL VEFFNKEGKY IEASLTANKS TNIEGKVIRC FFFLQIINKE SGLSCPELKE SAQS----LN
stB  GGQ-DTDKFP FSFFDRNGKY VQALLTRNKR VNMEGDTIGA FCFIQIASPE LQQALRVQRQ QEKKCYSQMK
osB  GGQ-DCEKFP FSFFDKNGKY VQALLTANTR SRMDGEAIGA FCFLQIASPE LQQAFEIQRH HEKKCYARMK
atC  SGQKNIEKLL FGFYHRDGSF IEALLSANKR TDIEGKVTGV LCFLQVPSPE LQYALQVQQI SEHAIACALN
ANG  ---------- --F------- ----L----- ----G----- --F------- ---------- ----------
CON  ---------- --F------- ----L----- ----G----- --F------- ---------- ----------

                                                                  INTRON
              970        980        990       1000       1010       1020     1030
                *          *          *          *          *          * |        *
sm   ELAYIRQEIK NPLYGIMFTR TLMETTDLSK DQKQYFETGA VCEKQIRKIL DDMDLESIED G--YLELDTT
cp   ---------- ---------- ---------- ---------- ---------- ---------- ----------
ac   ELSYVKEELK KPLEGLAFTR TVLEGTNLTI EQRQLIKTNA WCERQLRKIL -EDDLNNIEE G--YMDLEMS
atA  ALAYIKRQIR NPLSGIMFTR KMIEGTELGP EQRRILQTSA LCQKQLSKIL DDSDLESIIE G--CLDLEMK
cpA  ALGYIKRQIQ NPLSGIIFSR RLLERTELGV EQKELLRTSG LCQKQISKVL DESDIDKIID G--FIDLEMD
psA  VLTYMKRQIR NPLAGIVFSS KMLEGTDLET EQKRIVNTSS QCQRQLSKIL DDSDLDGIID G--YLDLEMA
stA  VLAYIRRQIR NPLSGIIFSR KMLEGTSLGE EQKNILHTSA QCQRQLDKIL DDTDLDSIIE G--YLDLEML
asA  AFSYMRHAIN NPLSGMLYSR KALKNTDLNE EQMKQIHVGD NCHHQINKIL ADLDQDSITE KSSCLDLEMA
osA  AYSYMRHAIN NPLSGMLYSR KALKNTGLNE EQMKEVNVAD SCHRQLNKIL SDLDQDSVMN KSSCLDLEMV
zmA  AFSYMRHAIN KPLSGMLYSR ETLKSTGLNE EQMRQVRVGD NCHRQLNKIL ADLDQDNITD KSSCLDLDMA
atB  ELAYICQVIK NPLSGMRFAN SLLEATDLNE DQKQLLETSV SCEKQISRIV GDMDLESIED G--SFVLKRE
atD  ELAYIFQVIK NPLSGLRFTN SLLEDMDLNE DQKQLLETSV SCEKQISKIV GDMDVKSIDD G--SFLLERT
atE  ELTYVRQEIK NPLNGIRFAH KLLESSEISA SQRQFLETSD ACEKQITTII ESTDLKSIEE G--KLQLETE
stB  ELAYICQEIK SPLNGIRFTN SLLEATNLTE NQKQYLETSA ACERQMSKII RDIDLENIED G--SLTLEKE
osB  ELAYIYQEIK NPLNGIRFTN SLLEMTDLKD DQRQFLETST ACEKQMSKIV KDASLQSIED G--SLVLEKG
atC  KLAYLRHEVK DPEKAISFLQ DLLHSSGLSE DQKRLLRTSV LCREQLAKVI SDSDIEGIEE G--YVELDCS
ANG  ---Y------ -P-------- ---------- -Q-------- -C--Q----- ---------- ------L---
CON  ---Y------ -P-------- ---------- -Q-------- -C--Q----- ---------- ------L---
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APPENDIX 1. Continued.

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            1040        1050        1060        1070        1080        1090        1100
               *           *           *           *           *           *           *
sm   EFMMGTVMDA VISQGMITSK EKNLQLIRET PKEIKAMFLY GDQVRLQQVL ADFLLNAIRF TPSSEN----
cp   ---------- ---------- ---------- ---------- ---------- ---------- ----------
ac   EFFMGSVIDA VISQGMAASR GKGVQILTEI PNDVKLMCLF GDQARLQQVL ADLLFCAINH ATTTNEDEKD
atA  EFTLNEVLTA STSQVMMKSN GKSVRITNET GEEVMSDTLY GDSIRLQQVL ADFMLMAVNF TPSGG-----
cpA  EFTLHEVLMV SISQVMLKIK GKGIQIVNET PEEAMSETLY GDSLRLQQVL ADFLLISVSY APSGG-----
psA  EFTLHEVLVT SLSQVMNRSN TKGIRIANDV AEHIARETLY GDSLRLQQVL ADFLLISINS TPNGG-----
stA  EFKLHEVLVA SISQVMMKSN GKNIMISNDM VEDLLNETLY GDSPRLQQVL ANFLLVSVNS TPSGG-----
asA  EFLLQDVVVA AVSQVLITCQ GKGIRISCNL PERFMKQSVY GDGVRLQQIL SDFLFISVKF SPVGG-----
osA  EFVLQDVFVA AVSQVLITCQ GKGIRVSCNL PERYMKQTVY GDGVRLQQIL SDFLFVSVKF SPVGG-----
zmA  EFVLQDVVVS AVSQVLIGCQ AKGIRVACNL PERSMKQKVY GDGIRLQQIV SDFLFVSVKF SPAGG-----
atB  EFFLGSVINA IVSQAMFLLR DRGLQLIRDI PEEIKSIEVF GDQIRIQQLL AEFLLSIIRY APSQE-----
atD  EFFIGNVTNA VVSQVMLVVR ERNLQLIRNI PTEVKSMAVY GDQIRLQQVL AEFLLSIVRY APMEG-----
atE  EFRLENILDT IISQVMIILR ERNSQLRVEV AEEIKTLPLN GDRVKLQLIL ADLLRNIVNH APFPNS----
stB  DFFLGSVIDA VVSQVMLLLR EKGVQLIRDI PEEIKTLTVH GDQVRIQQVL ADFLLNMVRY APSPDG----
osB  EFSLGSVMNA VVSQVMIQLR ERDLQLIRDI PDEIKEASAY GDQYRIQQVL CDFLLSMVRF APAENG----
atC  EFGLQESLEA VVKQVMELSI ERKVQISCDY PQEVSSMRLY GDNLRLQQIL SETLLSSIRF TPALRGL---
ANG  -F-------- ---Q------ ---------- ---------- GD----Q--- ---------- -P--------
CON  -F-------- ---Q------ ---------- ---------- GD----Q--- ---------- ----------

            1110        1120  INTRON 1130        1140        1150        1160        1170
               *           *      |    *           *           *           *           *
sm   WVGIKVATSR KRLGGVVHVM HLEFRITHPG VGLPEELVQE MFDRGRGM-T QEGLGLSMCR KLVKLMN-GE
cp   ---------- ---------- ---------- ---------- ---------- ---------- ----------
ac   WVTIKVSRTK TRLDDGVHLM HFESRISHSG QGISEALVEE MTNKSQKW-T PEGLAISISC TLIRLMN-GD
atA  QLTVSASLRK DQLGRSVHLA NLEIRLTHTG AGIPEFLLNQ MFGTEE-DVS EEGLSLMVSR KLVKLMN-GD
cpA  QLTISTDVTK NQLGKSVHLV HLEFRITYAG GGIPESLLNE MFGSEE-DAS EEGFSLLISR KLVKLMN-GD
psA  QVVIAASLTK EQLGKSVHLV NLELSITHGG SGVPEAALNQ MFGNNV-LES EEGISLHISR KLLKLMN-GD
stA  KLSISGKLTK DRIGESVQLA LLEFRIRHTG GGVPEELLSQ MFGSEA-DAS EEGISLLVSR KLVKLMN-GE
asA  SVEISSKLTK NSIGENLHLI DLELRIKHQG LGVPAELMAQ MFEEDNKEQS EEGLSLLVSR NLLRLMN-GD
osA  SVEISCSLTK NSIGENLHLI DLELRIKHQG KGVPADLLSQ MYEDDNKEQS DEGMSLAVSR NLLRLMN-GD
zmA  SVDISSKLTK NSIGENLHLI DFELRIKHRG AGVPAEILSQ MYEEDNKEQS EEGFSLAVSR NLLRLMN-GD
atB  WVEIHLSQLS KQMADGFAAI RTEFRMACPG EGLPPELVRD MFHSSR-WTS PEGLGLSVCR KILKLMN-GE
atD  SVELHLCPTL NQMADGFSAV RLEFRMACAG EGVPPEKVQD MFHSSR-WTS PEGLGLSVCR KILKLMN-GG
atE  WVGISISPGQ ELSRDNGSRI HLQFRMIHPG KGLPSEMLSD MFETRDGWVT PDGLGLKLSR KLLEQMN-GR
stB  WVEIQLRPSM MPISDGVTVV HIELGLYAPG -RLPPELVQD MFHSSR-WVT QEGLGLSMCR KMLKLMN-GE
osB  WVEIQVRPNI KQNSDGTDTM LFPFRFACPG EGLPPEIVQD MFSNSR-WTT QEGIGLSICR KILKLMG-GE
atC  CVSFKVIARI EAIGKRMKRV ELEFRIIHPA PGLPEDLVRE MFQPLRKGTS REGLGLHITQ KLVKLMERGT
ANG  ---------- ---------- ---------- ---P------ M--------- --G--L---- -----M----
CON  ---------- ---------- ---------- ---------- M--------- --G------- -----M----

            1180        1190        1200        1210
               *           *           *           *
sm   VEYIREAGKN YFLVSLELPL AQRDDAGSVK FQASS----- -
cp   ---------- ---------- ---------- ---------- -
ac   VKYTTDAGNK CFLVTIQFPL AHRDDATSVR ---------- -
atA  VQYLRQAGKS SFIITAELAA ANK------- ---------- -
cpA  VRYMREAGKS SFIITVELAA AHKSRTT--- ---------- -
psA  VRYLKEAGKS SFILSVELAA AHKLKG---- ---------- -
stA  VQYLREAGRS TFIISVELAV ATKSS----- ---------- -
asA  VRHLREAGVS TFIITAELAS APTAMGQ--- ---------- -
osA  VRHMREAGMS TFILSVELAS APAK------ ---------- -
zmA  IRHLREAGMS TFILTAELAA APSAVGR--- ---------- -
atB  VQYIRESERS YFLIILELPV PRKRPLSTAS GSGDMMLMMP Y
atD  VQYIREFERS YFLIVIELPV PLMMMMPSS- ---------- -
atE  VSYVREDERC FFQVDLQVKT MLGVESRGTE GSSSIK---- -
stB  IQYIRESERC YFLIILDLPM TRKGPKSVG- ---------- -
osB  VQYIRESERS FFHIVLELPQ PQQAASRGTS ---------- -
atC  LRYLRESEMS AFVILTEFPL I--------- ---------- -
ANG  ---------- -F-------- ---------- ---------- -
CON  ---------- -F-------- ---------- ----------
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Appendix 1. Continued.

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                 300       310       320       330       340       350       360
                  *         *         *         *         *         *         *
Mougeotia[1]    DEHGEVVAEIRRSDLEPYLGLHYPATDIPQASRFLFIKNRIRMICDCTSPQVKVVQDSRIPQEMS
Ceratodon[2]    DEHGEVVAEIRRMDLEPYMGLHYPATDIPQASRFLLMKNRVRLIADCYASPVKLIQDPDIRQPVS
Selaginella[3]  DEHGEVVAEIRRSDLEPYLGLHYPATDIPQASRFLFMKNRVRMICDCSAPPVKITQDKELRQPIS
Psilotum[3]     DEHGEVVAEIRRSDLEPFVGIHYPATDIPQACRFLFLKNRVTMICDCYAPPIRIIQDRQLKQPLS
Adiantum[4]     DEHGEVVAEIRRTDLEPYIGLGYPATDIPQAARFLFMKNRVRMICDCRLPPVKLIQDKTLSQPMS
Anemia[5]       DEHGEVVAEIRRSDLEPYMGLHYPATDIPQAARFLFMKNRVRLIYDCRLPPVKVIQDKNLVQPLS
Dryopteris[5]   DEHGEVLAEIRRSDLEPYLGLHYPATDIPQASRFLFMKNRVRMICDCRAIPVRVIQDKELRQPLS

                      370       380       390       400       410       420
                       *         *         *         *         *         *
Mougeotia       LAGSTMRGVHGCHTQYMMNMGSTASLVMCVTINDTNE------IAGGPGMKGRKLWGLIVCHHST
Ceratodon       LAGSTLRAPHGCHAQYMGNMGSIASLVMAVIINDNEE------YSRGAIQRGRKLWGLVVCQHTS
Selaginella     LAGSTLRAPHGCHAQYMGNMGSVASLVMAMIINDNDE--PSGGGGGGQHKGRRLWGLVVCHHTS
Psilotum        LAGSTLRAPHGCHAHYMGNMGSIASLVMAVIVKRHGEED---RSLGFQSQNGNRLWGMVVCHHTT
Adiantum        LTGSTLRAPHGCHTQYMANMNSISSLVMAVIVNDSDDD------SAGHSSQGIKLWGLVVCHHTS
Anemia          LAGSTLRAPHRCHAEYMGNMGSIASLGMAVIVNDDDSSD------AGNMQQRTRLWGLVVCHHTS
Dryopteris      LAGSTLRAPHGCHGQYMANMGSIASLVMAVVVNDNDED----LSNRPHQPKMRRLWGLVVCHHTT

                 430       440       450       460       470       480       490
                  *         *         *         *         *         *         *
Mougeotia       PRHIPFPIHSACEFLMQVFGLQLNMEAELAAQHREKHILRTQTLLCDMLLRDA-PMGIVSQSPN
Ceratodon       PRTVPFPLRSVCEFLMQVFGMQLNLHVELAAQLREKHILRTQTLLCDMLLRDA-PIGIVSQTPN
Selaginella     PRSVPF-LRSACEFLMQVFGLQLNMEAAVAAHVREKHILRTQTLLCDMLLRDA-PIGIVSQSPN
Psilotum        PRAVPFALRCACEFFAQVFALQLNMELELAAQMREKDILRTQSLLCDMLLRDA-PIGIVTRSPN
Adiantum        PRYVPFPVRSACEFLMQVFSLQLNMEVGMAAQVREKHILRTQTLLCDMLLRDA-PIGIVSQSPN
Anemia          TRYVPFPLRSACEFLMQVFSLELNMEVELAAQRREKHILQTQTLLCDMLLRDA-PIGIVSQSPN
Dryopteris      PRAVPFALRSACEFLMQVFGLQINMELELAAQMREKHILRTQTLLCDMLLRDA-PIGIVSESPN
```

APPENDIX 2. Regions of all nonangiosperm amino acid sequences available that are homologous with the *Mougeotia* gene fragment, numbered with reference to Appendix 1. [1]Winands et al. (1992); [2]Thümmler et al. (1992); [3]Hanelt et al. (1992); [4]Okamoto et al. (1993); [5]Maucher et al. (1992).

APPENDIX 3. Sources of *PHY* sequences determined in this study. Arrangement of flowering plants follows Cronquist (1981) and Polhill (1994).

Subclass/Tribe	Species	Source/Voucher
Sphenophyta	*Equisetum arvense* L.	P. Soltis (no voucher)
Pinophyta	*Gingko biloba* L.	*S. Mathews 365* MONT
	Pseudotsuga menziesii (Mirb.) Franco	*S. Mathews s.n.* MONT
Magnoliophyta		
Monocots		
Alismatidae	*Elodea* Michx. sp.	S. Mathews (no voucher)
Arecidae	*Lemna gibba* L.	J. Silverthorne (no voucher)
Commelinidae	*Hordeum vulgare* L.	*S. Mathews s.n.* MONT
	Calamovilfa longifolia (Hook.) Scribn.	*Lavin s.n.* MONT
	Panicum capillare L.	*Lavin s.n.* MONT
Zingiberidae	*Billbergia nutans* H. Wendl	*S. Mathews 351* MONT
Liliidae	*Muscari* Mill. sp.	S. Mathews (no voucher)
Dicots		
Magnoliidae	*Ceratophyllum demersum* L.	*S. Mathews s.n.* MONT
	Aquilegia L. sp.	S. Mathews (no voucher)
Hamamelidae	*Urtica dioca* L.	*S. Mathews 330* MONT
	Quercus turbinella Greene	*J. M. Tucker 4491* UCD
Caryophyllidae	*Dianthus caryophyllus* L.	R. Woodson (no voucher)
	Spinacia oleracea L.	S. Mathews (no voucher)
Dilleniidae	*Arabidopsis thaliana* (L.) Schur	S. Mathews (no voucher)
Asteridae	*Lycopersicon esculentum* Mill.	S. Mathews (no voucher)
	Antirrhinum majus L.	*S. Mathews 301* MONT
Rosidae	*Daucus carota* L.	S. Mathews (no voucher)
Fabaceae		
Dalbergieae	*Dalbergia* L.f. sp.	*Lavin 7141* MONT
	Tipuana tipu (Benth.) Kuntze	*Lavin 6184* BH
Galegeae	*Caragana arborescens* Lam.	*Lavin 5907* RM
	Clianthus formosus (G. Don) Ford & Vick	Krukoff s.n. K
Millettieae	*Dalbergiella nyasae* Baker f.	*Muller 2686* K
	Derris elliptica (Wallich) Benth.	Michigan State Univ. Conservatory (no voucher)
	Kunstleria blackii (F. Muell.) Prain	*Pedley 5005* K
	Lonchocarpus eriocarinalis Micheli	*Lavin 5325a* BH
	Millettia dura Dunn	*Lock 83/124* K
	Millettia richardiana (Baill.) D. J. Du Puy & J. Labat	*Schrire et al. 2555* K
	Piscidia piscipula (L.) Sarg.	*Lavin & Luckow 5793a* TEX
	Wisteria floribunda (Willd.) DC	*Lavin 6205* BH
	Xeroderris stuhlmanii (Taub.) Mendonca & E. P. Sousa	*Corby 2162* K
Robinieae	*Hebestigma cubense* (HBK) Urb.	*Lavin 5611* TEX
	Lennea melanocarpa (Schltdl.) Vatke ex Harms	*Lavin & Delgado 8217* MEXU
	Sesbania sesban (L.) Merr.	*Potter 870410* BH
	Sesbania vesicaria (Jacq.) Elliot	*Lavin s.n.* TEX
Sophoreae	*Myrospermum sousanum* A. Delgado & M. C. Johnston	*Delgado & Johnston s.n.* TEX
Vicieae	*Lathyrus odoratus* L.	*Lavin 6170* MONT

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                  330       340       350       360       370       380       390       400                410
                   *         *         *         *         *         *         *         *                  *
Myrospermum B   QASRFLFKQNRVRMIVDCNATPVRVIQDERLRQPLCLVGSTLRAPHGCHAQYMQKMGSIASLVMAVIINGND--EEAV-GG-----RSSMKLWGLVVCHH
Xeroderris B    QASRFLFKQNRVRMIVDCHASPVSVVQDEALVQPLCLVGSTLRAPHGCHAQYMANMGSIASLVMAVIINGND--EEGV-GG-----RSSMRLWGLVVCHH

Myrospermum A   QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVLAVVVNDSD--EDGD-SSDAVQPQKRKRLWGLVVCHN
Poitea A        QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLAFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SSDAIQPQKRKRLWGLVVCHN
Hybosema A      QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SSDAVQPQKRKRLWGLVVCHN
Hebestigma A    QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SSDAVQPQKRKRLWGLVVCHN
Lennea A        QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SSDAVQPQKRKRLWGLVVCHN
Sesbanias A     QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SSDAVQPQKRKRLWGLVVCHN
Sesbaniav A     QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SSDAVQPQKRKRLWGLVVCHN
Piscidia A      QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDNE--ENGD-GSDAVQPQKRKRLWGLVVCHN
Kunsteria A     QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMNSIASLVLAVVVNDNE--EDGD-SSDAVQPQKRKRLWGLVVCHN
Dalbergiella A  QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPLDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDNE--EDGD-SSDAVQPQKRKRLWGLVVCHN
Dalbergia A     QASRFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHSQYMANMDSIASLVMAVIVNDND--EDGD-SSDAVQPQKRKRLWGLVVCHN
Tipuana A       QASRFLFMKNKVRMIVDCHAKHVKILQNEKLPFDLTLCGSTFRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SSDAVQPQKRKRLWGLVVCHN
Xeroderris A    QAARFLFMKNKVRIIVDCHAKHVKVLQDEKLPLDLTLCGSTLRAPHSCHLQYMANMDSIASLFMAVVVNDNE--EDGD-SSDAVQPQKRKRLWGLVVCHN
Millettiar A    QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDNE--EDGD-SADAVQPQKRKRLWGLVVCHN
Millettiad A    QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDNE--EDGD-SSDAVQPQKRKRLWGLVVCHN
Lonchocarpus A  QAARFLFMKNKVRMIVDCHAKHVKVLQDEKIPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDNE--EDGD-SSDAVQPQKRKRLWGLVVCHN
Derris A        QAARFLFMKNKVRMIVDCHAKHVKVLQDEKIPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDNE--EDGD-SSDAVQPQKRKRLWGLVVCHN
Caragana A      QAARFLFMKEQDPYIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMNSIASLVMAVVVNDSD--EDED-SSDAVHPQKRKRLWGLVVCHN
Pisum A         QAARFLFMKNKVRMIVDCNAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SADAVLPQKKKRLWGLVVCHN
Lathyrus A      QAARFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SRDAVLPQKKKRLWGLVVCHN
Wisteria A      QAARFLFMKEQGAYIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMDSIASLVMAVVVNDSD--EDGD-SPDAVHPQKRKRLWGLVVCHN
Clianthus A     TRSRFLFMKNKVRMIVDCHAKHVKVLQDEKLPFDLTLCGSTLRAPHSCHLQYMANMNSIASLVMAVVVNDSD--EDGDNSSDAVQPQKKKRLWGLVVCHN

Sesbanias A'   QATRFLFMKNKVRIIVDCSAKHVKVIQDKNIPFDLTLCGSTLRAPHSCHLQYMENMKASASLVMAVVVNDSN--EDGD-SSDAVQPQKRKRLWGLVVCHN
Sesbaniav A'   QATRFLFMKNKVRMIVECCAKHVKVIQDKKNPFDLTLCGSTLRAPHSCHLQYMENMKASASLVMAVVINDSN--EDGD-SSDAVQPQKRKRLWGLVVCHH
Poitea A'      QATRFLFMKNKVRMIVDCSANHVKVLQDKNIPFDLTFCGSTLRAPHSCHLQYMKNMNASASLVMAVVVNDSD--EDGD-SFDVVQLQKRRRLWGLDVCHH
Hybosema A'    QATRFLFMKNKVRMIVDCSANHVKVPQDKNIPFDLTFCGSTLRAPHSCHLQYMKNMNASASLVMAIVVNDSD--EDGD-SSDAVQLQNRRRLWGLVVCHH
Dalbergia A'   QATRFLFMKNRVRMIVDCRAKHVKVLQDLKIPFDLTLCGSTLRAAHSCHLQYMENMNSIASLVMAVVVNDDD--DDGD-GSDVVQPQKRKRLWGLVVCHN
Tipuana A'     QATRFLLMKNKVRMIVDCRAKHVKVLQDPKIPYDLTLCGSTLRAAHSCHLQYMENMNTIASLVMAVVVNDND--EDGD-GSDVVQPQKRKRLWGLVVCHN
Caragana A'    QATLFLFMKNKVHMMVDRRAKHVKVLQDQKIPFDLTLCGSTLRAPHSCHLQYMENMNTSASLVMAVVVNDSN--EDED-SSDDVQLQKRKRLWGLVVCHH
Wisteria A'    QATRFLFMRNKVRMIVDCRAKHVKVLQDKKIPFDLTFCGSTLRAPHSCHLQYMENMNASASLVLAVVVNDND--EDED-SSNDVQPQKRKRLWGLVVCHH
Piscidia A'    QATRFLFMKNKVRMIVDCRAKHVKVLQDEKVPFDLTLCGSTLRAPHSCHLQYMENMNCSASLVMAVVVNDND--EDGD-S-DAVQPQKRKRLWGLVVCHN
Millettiad A'  QATRFLFMKNKVRMIVDCRAKHVKVLQDKKVPFDLTLCGSTLRAPHSCHLQYMENMNCSASLVMAVVVNDND--EDGD-S-DAVQPQKRKRLWGLVVCHN
Lonchocarpus A'QATRFLFMKNKVRMIVDCRAKHVKVLQDKKVPFDLTLCGSTLRAPHSCHLQYMENMNCSASLVMAVVVNDND--EDGD-S-DAVQPQKRKRLWGLVVCHN

Myrospermum E   QAARFLFKQNRVRMICDCYAKPVKVIQSEELRRPLCLVNSTLRSPHGCHTQYMANMGSIASLVMAVTVNGNDTT----------------RLWGLLVCHH
Hebestigma E    QAARFLFKQNRVRMITDCHAKPVKVIQSEELRQPLCLVNSTLRSPHECHTQYMANMGSIASLVMAVIVNRNDTT----------------RLWGLLVCHH
Lennea E        QAARFLFKQNRVRMITDCNAKPVKVIQSEELKQPLCLVNSTLRSPHECHTQYMANMGSIASLVMAVIVNENDTT----------------RLWGLLVCHH
Dalbergia E     QASRFLFRQNRVRMICDCHAKPVKVFQSEELRQPLCLVNSTLRSPHGCHTQYMANMGSIASLVMAIIVNGKHTT----------------RLWGLLVCHH
Dalbergiella E  QAARFLFKQNRVRMICDCHAKPVKVIQSGELRQPLCLVNSTLRSPHGCHTQYMANMGSISSLVMAVVVNGNDTT----------------RLWGLLVCHH
Kunstleria E    QASRFLFKQNRVRMICDCHAKPVKVIQSEELRQPLCLVNSTLRSPHGCHTQYMANMGSIASLVMAVTVNGNDTR----------------RLWGLLVCHH
Xeroderris E    QACRFLFKQNRVRMICDCHAKPVKVIQSEELRQPLCLVNSTLRSPHGCHTQYMANMGSVASLVMAVIVNGNDTT----------------RLWGLLVCHH
Lonchocarpus E  HASRFLFKQNRVRMICDCDAKPVKVIQSEELRQPLCLVNSTLRSPHVCHTQYMANMGSTASLVLAIIVNGNDTT----------------RLWGLLVCHH
Derris E        QASRFLFKQNRVRMICDCHAKPVKVIQSEELRQPLCLVNSTLRSPHVCHTQYMANMGSIASLVMAIIVNGNDTT----------------RLWGLLVCHH
Millettiar E    QASRFLFKQNRVRMICDCHAKPVKVIQSEELRQPLCLVNSTLRSPHVCHTQYMANMGSIASLVMAIIVNGNDKT----------------RLWGLLVCHH
Millettiad E    QASRFLFKQNRVRMICDCNAKPVKVIQSEELRQPLCLVNSTLRSPHVCHTQYMANMGSLASLVMAIIVNGNDTT----------------RLWGLLVCHH
Piscidia E      QASRFLFRQNRVRMICDCHAKPVKVIQSEELRQPLCLVNSTLRSPHVCHTQYMSNMGSIASLVMAIIVNGNDKR----------------RLWGLLVCHH
Wisteria E      QASRFLFKQNRVRMICDCHAKPVKVIQSEELRQPLCLVNSTLRSPHDCHTHYMANMGSIASLVMAVIVNANDAP----------------RLWGLLVCHH
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APPENDIX 4. Aligned amino acid sequences inferred from nucleotide sequences amplified from the Fabaceae, with 6 C-terminal residues deleted. Amino acids are numbered relative to those in Appendix 1. Orthologous *Arabidopsis PHY* loci are indicated to the right of the name of the genus.

APPENDIX 5. Relative rate tests (Wu & Li, 1985) to detect rate asymmetry. * $P < 0.05$; ** $P < 0.01$. d_{13} and d_{23} are the number of nonsynonymous (or synonymous in legume comparisons) substitutions per site between species 1 and 3, and species 2 and 3, respectively; under the null hypothesis $d_{13} = d_{23}$. SE is standard error.

Species 1	Species 2	Species 3 (reference)	$d_{13} - d_{23} \pm$ SE
	Chromophore region only (330–594 bp) compared		
ArabidopsisA	CucurbitaA	Selaginella	0.0082 ± 0.0194
ArabidopsisA	SolanumA	Selaginella	0.0024 ± 0.0415
ArabidopsisA	OryzaA	Selaginella	−0.0033 ± 0.0416
ArabidopsisA	ArabidopsisB	Selaginella	0.0451 ± 0.0395
ArabidopsisA	ArabidopsisC	Selaginella	0.0761 ± 0.0379
ArabidopsisA	ArabidopsisD	Selaginella	0.0537 ± 0.0390
ArabidopsisA	ArabidopsisE	Selaginella	0.0709 ± 0.0387
SolanumA	SolanumB	Selginella	0.0847 ± 0.0373*
OryzaA	OryzaB	Selaginella	0.0639 ± 0.0387
AvenaA	ZeaA	Selaginella	−0.0081 ± 0.0161
MyrospermumA	HebestigmaA	PisumA	0.0661 ± 0.1679
MilletiaA	SesbaniaA	MyrospermumA	0.1337 ± 0.1350
PisumA	HebestigmaA	MyrospermumA	0.2012 ± 0.1466
HebestigmaE	MilletiaE	MyrospermumE	−0.0536 ± 0.1002
	N-Terminal encoding sequence (2400 bp) compared		
ArabidopsisA	CucurbitaA	Selaginella	0.0064 ± 0.0216
ArabidopsisA	SolanumA	Selaginella	−0.0107 ± 0.0220
ArabidopsisA	OryzaA	Selaginella	0.0053 ± 0.0216
ArabidopsisA	ArabidopsisB	Selaginella	0.0272 ± 0.0253
ArabidopsisA	ArabidopsisC	Selaginella	−0.0115 ± 0.0214
ArabidopsisA	ArabidopsisD	Selaginella	0.0182 ± 0.0213
ArabidopsisA	ArabidopsisE	Selaginella	−0.0228 ± 0.0225
ArabidopsisB	ArabidopsisC	Selaginella	−0.0387 ± 0.0221
ArabidopsisB	ArabidopsisE	Selaginella	−0.0500 ± 0.0218*
ArabidopsisB	SolanumB	Selaginella	0.0346 ± 0.0195
ArabidopsisB	OryzaB	Selaginella	−0.0008 ± 0.0204
SolanumA	SolanumB	Selaginella	0.0725 ± 0.0205**
OryzaA	OryzaB	Selaginella	0.0211 ± 0.0210
	Full-length coding sequence (3384 bp) compared		
ArabidopsisA	CucurbitaA	PisumA	−0.0112 ± 0.0111
ArabidopsisA	SolanumA	Selaginella	−0.0145 ± 0.0199
ArabidopsisA	OryzaA	Selaginella	−0.0226 ± 0.0202
ArabidopsisA	ArabidopsisB	Selaginella	0.0476 ± 0.0187*
ArabidopsisA	ArabidopsisC	Selaginella	−0.0346 ± 0.0206
ArabidopsisA	ArabidopsisD	Selaginella	0.0339 ± 0.0190
ArabidopsisA	ArabidopsisE	Selaginella	−0.0273 ± 0.0204
ArabidopsisE	ArabidopsisB	Selaginella	0.0749 ± 0.0194**
ArabidopsisE	ArabidopsisD	Selaginella	0.0612 ± 0.0197**
ArabidopsisC	ArabidopsisB	Selaginella	0.0822 ± 0.0194**
ArabidopsisC	ArabidopsisD	Selaginella	0.0685 ± 0.0197**
ArabidopsisB	SolanumB	Selaginella	0.0326 ± 0.0234
ArabidopsisB	OryzaB	Selaginella	−0.0085 ± 0.0179
SolanumA	SolanumB	Selaginella	0.0947 ± 0.0183**
OryzaA	OryzaB	Selaginella	0.0617 ± 0.0194**

THE COMPARATIVE POLLINATION AND FLORAL BIOLOGY OF BAOBABS (*ADANSONIA*–BOMBACACEAE)[1]

David A. Baum[2]

ABSTRACT

The baobabs comprise eight species with large, spectacular, nocturnal flowers. The African baobab, *Adansonia digitata*, has long been known to be bat-pollinated. In this paper I document the floral biology and pollination systems of the remaining seven species. The two species in section *Brevitubae*, both endemic to Madagascar, are pollinated by nocturnal mammals (fruit bats and lemurs). In contrast, the five species in section *Longitubae*, four endemic to Madagascar and one to Australia, are pollinated by long-tongued hawkmoths. In all cases, animals besides the legitimate pollinators also exploited nectar and pollen. The two pollination systems occurring in the genus correlate closely with differences in the floral morphology, phenology, and nectar production.

The baobabs comprise eight species in the genus *Adansonia* L. (Bombacaceae), six endemic to Madagascar, one to northwestern Australia, and one originally from continental Africa that has been dispersed by humans elsewhere in the tropics (Wickens, 1983). They are tropical trees growing in savanna, deciduous forest, or, rarely, moist, semi-evergreen forest. The genus is characterized by massive, often bottle-shaped trunks, palmately compound leaves, and a large, dry, indehiscent fruit containing reniform seeds embedded in an edible pulp. All species of *Adansonia* have large, spectacular flowers, but there are great differences in their floral biology. This variation is partially reflected in the subgeneric classification, with the three sections differing in the shape of the floral bud, orientation of the flower, and length of the staminal tube (Hochreutiner, 1908; Baum, 1995). The African baobab (*A. digitata* L.) is the sole representative of section *Adansonia*. Two Malagasy species (*A. grandidieri* Baill. and *A. suarezensis* H. Perr.) constitute section *Brevitubae*. The Australian species (*A. gibbosa* (A. Cunn.) Baum ex Guymer) and four Malagasy species (*A. rubrostipa* Jumm. & H. Perr., *A. madagascariensis* Baill., *A. za* Baill., and *A. perrieri* Capuron) constitute section *Longitubae*.

In the early part of this century, African baobabs growing in botanical gardens in the Far East were used to support the then heterodox assertion that tropical bats were important pollinators of some tropical plants. Van der Pijl (1934) inferred from the descriptions of van Harreveld-Lako (1926) that *A. digitata* was bat-pollinated; this prediction was

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confirmed in Java by Porsch (1935) and van der Pijl (1936). Ten years later Jaeger (1945, 1950, 1954) completed the first study of bat-pollination in natural populations of *A. digitata* in West Africa. He observed and carefully described the floral morphology and course of anthesis and recorded the visits of fruit bats (*Eidolon helvum* Kerr) to the flowers. Jaeger's thorough work demonstrating bat-pollination in *A. digitata* has been confirmed by many subsequent studies (e.g., Harris & Baker, 1959; Start, 1972; Ayensu, 1974), with three species of fruit bat (*Eidolon helvum*, *Epomorphorus gambianus* Ogilby, and *Rousettus aegyptiacus* E. Geoffroy) identified as major pollinators.

Agents other than fruit bats have been suggested to play a role in the pollination of *A. digitata*. Jaeger (1945) proposed wind-pollination, but this is unlikely because the pollen is not particularly light and the stigmatic area is small. In addition, bushbabies (*Otolemur crassicaudatus* E. Geoffroy and *Galago senegalensis* E. Geoffroy) visit *A. digitata* and could contribute somewhat to pollination (Coe & Isaac, 1965; Wickens, 1983). However, they are infrequent visitors and are destructive to flowers (Wickens, 1983), so their net effect on reproductive output is likely to be negative. Although it is possible that ants steal nectar, Humphries (1982) is mistaken in suggesting that *A. digitata* is ant-pollinated.

In contrast to the extensive information on the pollination of *A. digitata*, the Malagasy and Australian species are very poorly known. Little fieldwork has been undertaken prior to this study and no nocturnal observations have been reported. Nonetheless, several workers have made predictions about the pollination of the other baobabs. Van der Pijl (1956) and J. Armstrong (1979) assumed that, like *A. digitata*, the other baobabs would prove to be bat-pollinated. However, the striking differences in the floral morphology of *A. digitata* from the rest of the genus make this inference questionable. Werth (1915) suggested that *A. madagascariensis* was bird-pollinated. Similarly, Patrick Armstrong (1983) argued against bat-pollination in *A. gibbosa* and, having observed visits by birds (P. Armstrong, 1977), suggested that it and all the Malagasy baobabs might be bird-pollinated. This view seemed to gain support from reports of red and yellow flowers in the Malagasy species, these colors being typical of ornithophilous flowers (Faegri & van der Pijl, 1979). However, he made no nocturnal observation of *A. gibbosa* and was unable to study any of the Malagasy species in the field (P. Armstrong, pers. comm.). Here I report on the results of extensive field studies aimed at documenting the pollination and floral biology of the baobabs of Madagascar and Australia.

Methods and Materials

The fieldwork was carried out during the course of four trips to Madagascar and one to Australia between October 1987 and December 1991. A brief trip to Kenya in January 1989 allowed observation of bat visits to *A. digitata*. The dates and locations of the work are given in Table 1. Figure 1 shows the distribution of the main study sites in Madagascar. At each site I studied the floral biology of 2–25 trees and made pollination observations on 2–5. I was careful to select trees near the center rather than at the periphery of the population and ones that had abundant, accessible flowers. Throughout my work access to the canopy was achieved using the method described by Perry (1978).

Floral biology

Most floral traits were scored on the basis of simple observation and measurement of fresh flowers. Here I will present only those characters that potentially influence pollination; general floral characteristics are described elsewhere (Baum, 1995).

Phenological data were derived from field observations and herbarium labels. In the case of *A. rubrostipa*, a more detailed study of 25 trees visible from a trail in Kirindy Forest was conducted. These trees were revisited daily for 15 days (February 10–25) and the number of freshly opened flowers recorded.

Nectar was extracted from flowers and its volume measured using microcapillary tubes. In sections *Adansonia* and *Brevitubae* the nectar was easily accessible. In section *Longitubae* the capillary tubes were carefully inserted between the petal bases. Unless otherwise stated, flowers were bagged from anthesis until the last nectar sample was taken.

The time course of nectar production was determined for *A. rubrostipa*, *A. grandidieri*, *A. za*, *A. perrieri*, and *A. gibbosa*. Initially, this was completed by making repeated measurements from the same flowers. This worked successfully with *A. grandidieri*, but when used on *A. rubrostipa* it seemed to damage the nectariferous tissue. To avoid these problems, in the three other species (*A. gibbosa*, *A. za*, and *A. perrieri*) samples of flowers were collected at each time interval and nectar was extracted destructively.

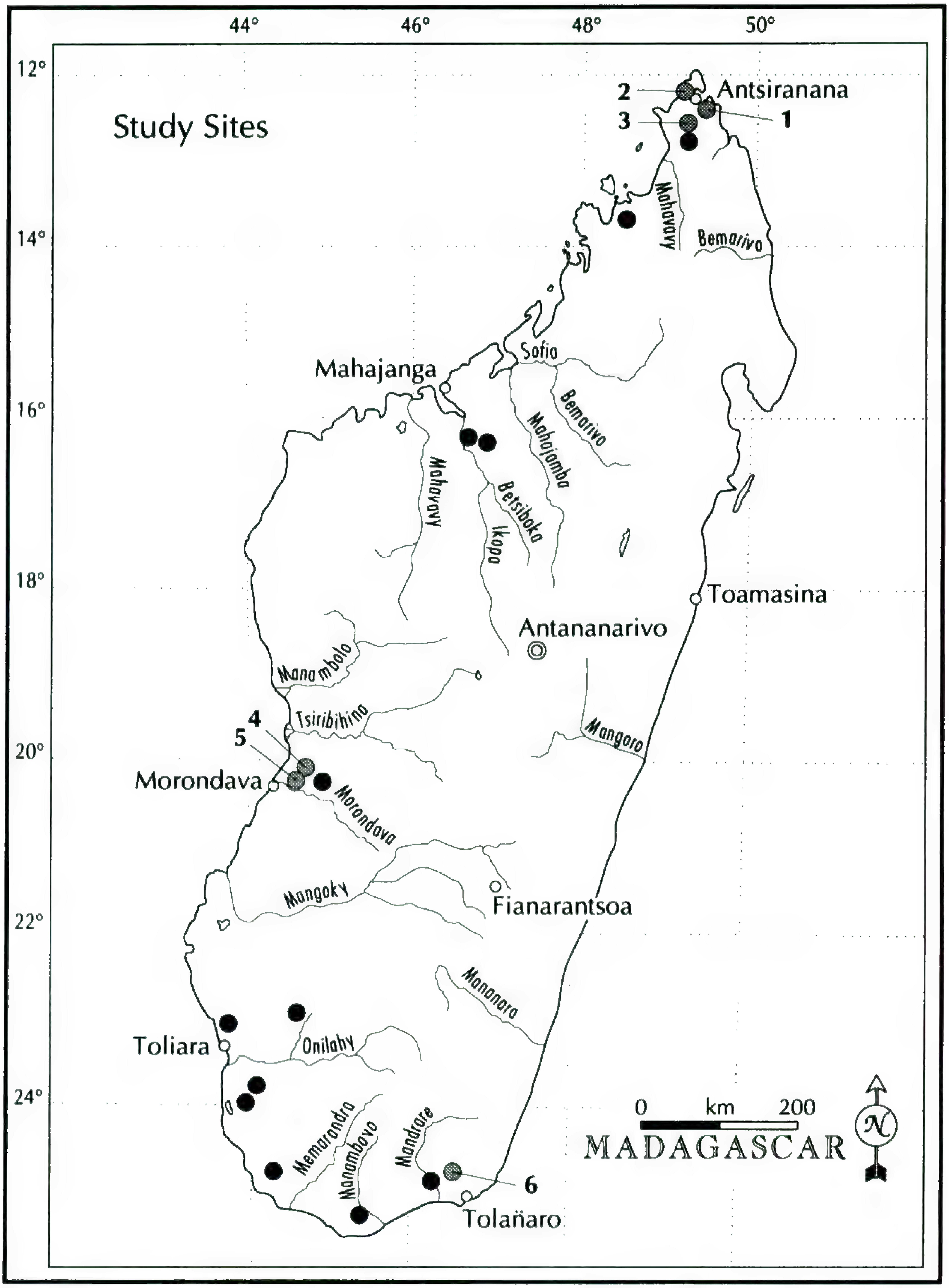


FIGURE 1. Study sites in Madagascar. Minor study sites are marked with a solid circle. Main study sites are numbered: (1) Montagnes des Français; (2) Beantely; (3) Montagne d'Ambre; (4) Kirindy Forest; (5) Marofandelia; (6) Andohahela.

TABLE 1. Dates and study sites. All localities are in Madagascar except for *Adansonia gibbosa*, which was studied in northwestern Australia (WA). For other collection localities, see Baum 1995.

Species	Dates	Locality
A. grandidieri	3–15 July 1989	Marofandelia near Morondava
A. suarezensis	6–15 June 1989	Montagnes des Français near Antisiranana
	20–22 June 1989	Beantely near Antsiranana
A. gibbosa	24 Nov.–1 Dec. 1989	Oscar ranges near Fitzroy Crossing, WA
	13–22 Dec. 1989	Meda Station near Derby, WA
	22 Dec. 1989–2 Jan. 1990	Yeeda Station near Derby, WA
A. rubrostipa	1–25 Feb. 1989	Kirindy forest near Morondava
A. madagascariensis	14–21 Mar. 1989	Montagnes de Français near Antsiranana
A. za	20–21 Dec. 1987	Andohahela near Tolagnaro (Fort-Dauphin)
	8–12 Dec. 1988	Kirindy forest near Morondava
	17–24 Dec. 1991	Kirindy forest near Morondava
A. perrieri	12–18 Nov. 1988	Montagne d'Ambre near Antsiranana
	20 Nov.–3 Dec.1991	Montagne d'Ambre near Antsiranana

Nectar concentration was measured using a temperature-compensated, hand-held refractometer (Reichert model 10431). Nectar samples, taken by letting drops of nectar dry on filter paper (Whatman #1), were analyzed by H. and I. Baker (University of California, Berkeley) for the presence of amino acids and to determine the sucrose/hexose ratio (see Baker & Baker, 1975, 1982, 1983).

In order to evaluate changes in receptivity, stigmatic morphology was examined throughout the night and day. The onset of receptivity was assessed using the peroxidase reaction. A drop of 5% hydrogen peroxide was placed on the stigma and observed with a hand-lens (magnification ×10). A positive reaction produced bubbles of oxygen. This test determines the onset of receptivity but cannot be used to detect the cessation of receptivity. In the case of *A. gibbosa*, there were significant levels of bird visitation in the early morning and it was of some importance to assess whether stigmas were receptive at that time. This was tested by emasculating buds prior to anthesis and bagging them throughout the night. These flowers were then pollinated soon after dawn with pollen from another tree in the same population. Control flowers were pollinated soon after anthesis. The fate of the flowers was followed for one month to observe whether or not abortion occurred. The breeding system experiment (see below) showed that in *A. gibbosa* most fruit abortion occurs within two weeks.

In the cases of *A. rubrostipa, A. madagascariensis, A. suarezensis, A. grandidieri,* and *A. gibbosa*, compatibility was assessed using pollen-tube growth. In the last species, fruit set was also measured to see if it accorded with the pollen-tube data. In both procedures, flowers were bagged during the day prior to anthesis with fine, mosquito-mesh bags. In *A. gibbosa*, the flowers were emasculated by removing the top of the calyx, opening the immature petals, and carefully cutting off all the filaments with a pair of scissors. In the other species, flowers were not emasculated. After anthesis, flowers were either selfed with pollen from the same tree or crossed with pollen from another tree.

Styles (styles and ovaries in the case of *A. gibbosa*) were collected 18 to 48 hours after pollination, fixed in 2 : 1 absolute ethanol : glacial acetic acid for 2–4 hours, and then stored in 70% ethanol. Pollen tube analysis was carried out in the laboratory of D. Mulcahy (University of Massachusetts, Amherst) using a protocol modified from Martin (1959). Styles were cleared in 8 N NaOH at 60°C for approximately 24 hr. They were rinsed in distilled water and placed in tris-glycine buffer (pH 8.4) for 15–20 min. The styles were stripped of hairs, then split longitudinally and placed with cut surfaces uppermost on a microscope slide with a drop of decolorized aniline blue (0.1% aniline blue (Allied Chemical Co.) in 0.1 M K_3PO_4). Ovaries were treated in the same way except that, after clearing, the placentas and ovules were dissected away from the ovary wall and placed on the slide. Cover slips were added and sealed with glycerin. Pollen tubes were observed using a Zeiss epifluorescence microscope at 160× and 400× magnification.

The fruit set experiment carried out on *A. gibbosa* followed the emasculation and pollination produced described above. In addition, some flowers were emasculated and bagged but left unpollinated to test for apomixis (cf. Baker, 1960). The polli-

nation bags were removed in the afternoon of the day after anthesis. Approximately two weeks later, the trees were revisited and the stage of maturation of the tagged fruit recorded.

POLLINATION

The flowers of *Adansonia* species are large, with the stigma, anthers, and nectar spatially separated. Hence, pollinators must be large-bodied and, therefore, behavioral observations and photographs were sufficient to infer whether floral visitors are likely to be major pollinators, minor pollinators, commensals, or floral parasites (see Baker et al., 1971). Observations were carried out either from the ground with binoculars or more usually from within the trees. Nocturnal observations were made using headlamps and flashlights with red filters. Photographs where taken mainly with a Pentax Super A camera and a Vivitar 80–210 mm zoom lens. At night, a small red flashlight was strapped to the lens to facilitate focusing, and a dedicated through-the-lens metered flash was used for illumination.

Animal visitors were identified in the field or from photographs. In addition, some hawkmoths were collected at flowers using a butterfly net. Malagasy specimens were deposited with B. Walther and L. Wasserthal of the Friedrich-Alexander-Universität, Erlangen-Nürnberg, Germany. Australian specimens were deposited at the Department of Entomology, Conservation and Land-management Service, South Perth, Western Australia. Hawkmoths were identified by E. Edwards, L. A. Nilsson, L. Wasserthal, and B. Walther and bats by K. Dobat.

RESULTS

FLORAL BIOLOGY

(1) Morphology. Morphological features are compared in Table 2, and Figure 2 shows a representative flower of each species. Some explanatory notes follow.

The crowns of section *Brevitubae* are distinguished from the rest of the genus by their flat-topped, "pagoda" form. The branches are tiered and more or less horizontal and the flowers are borne at the tips of orthotropic twigs. The remaining species have disorganized branches and a rounded crown.

The flowers of all *Adansonia* are borne on sturdy flower stalks comprising a proximal peduncle and a distal pedicel. The flower stalk in section *Brevitubae* and section *Longitubae* is short and either erect or more or less horizontal. In contrast, *A. digitata* (sect. *Adansonia*) has flowers that are pendulous on stalks up to 50 cm long.

Bud shape is unlikely to have any direct effect on pollination, and is probably a by-product of the development of other floral parts. In particular, petal length and width, the length of the androecium and gynoecium, and filament number are likely to affect bud shape. Baobabs have five calyx lobes, which enclose the flower completely in the bud (contrasting with the truncate calyx in all other genera of Bombacaceae). Prior to anthesis, the calyx lobes are fused along their entire length. They split apart during anthesis, curl outwards, and ultimately become twisted at the base of the flower (Fig. 2A, B, D–H). In *A. digitata* the calyx lobes reflex but do not twist (Fig. 2C). In sections *Longitubae* and *Brevitubae*, they frequently fail to separate completely resulting in a somewhat deformed flower. *Adansonia gibbosa* is unusual in the genus in that the corolla pushes through the tip of the calyx as much as 12 hours before anthesis. In the other species the corolla does not become visible until at most one hour before anthesis.

In sections *Adansonia* and *Brevitubae*, the petals and androecium more or less cover the calyx lobes in the open flower (Fig. 2A–C). In section *Longitubae*, the long staminal tube and upright petals cause the inner surface of the calyx to be exposed (Fig. 2D–H). In *A. gibbosa* this surface is cream-colored and villose like that of sections *Brevitubae* and *Adansonia* (Fig. 2A–D). In contrast, the inner calyx of *A. rubrostipa*, *A. madagascariensis*, and *A. za* is dark red (Fig. 2E–G). *Adansonia perrieri* shows intraspecific variation, with individual trees having the inner surface of the calyx pink or whitish.

Nectar is produced by a ring of calyx tissue around the base of the ovary. In *A. digitata*, the calyx tube is more or less flat and the nectar accumulates in drops on its hairy, inner (adaxial)

→

FIGURE 2. Comparison of the flowers of *Adansonia* species. The photographs show approximate relative sizes: see Table 2 for actual dimensions.—A. *A. grandidieri*.—B. *A. suarezensis*.—C. *A. digitata*.—D. *A. gibbosa*.—E. *A. rubrostipa*.—F. *A. madagascariensis* (red-petaled individual).—G. *A. za*.—H. *A. perrieri* (individual with whitish inner calyx).

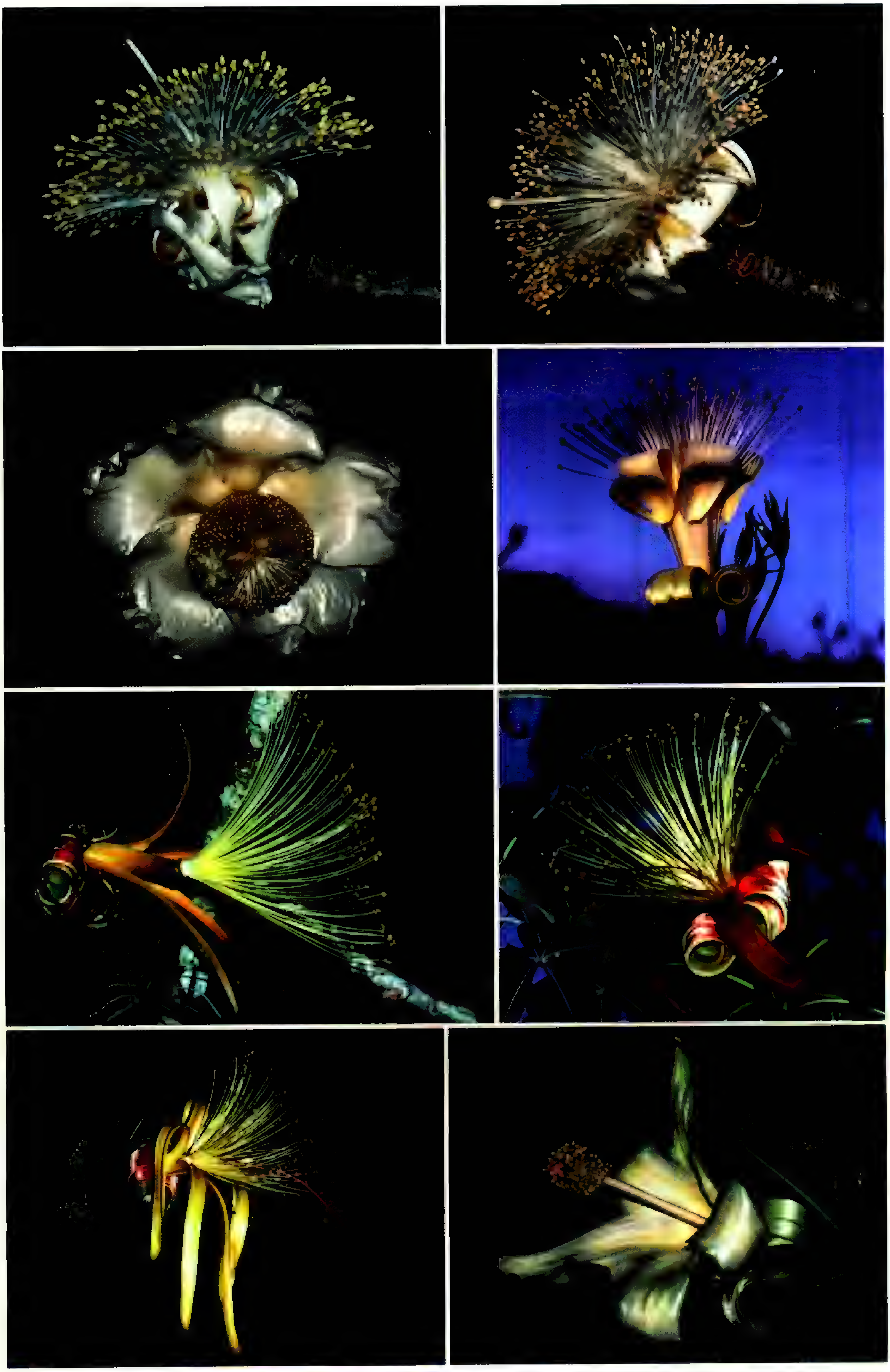


TABLE 2. The floral biology of *Adansonia*.

Character	Section *Brevitubae*		Section *Adansonia*	Section *Longitubae*				
	A. grandidieri	*A. suarezensis*	*A. digitata*	*A. gibbosa*	*A. rubrostipa*	*A. madagascariensis*	*A. za*	*A. perrieri*
Morphology								
Crown form	flat-topped	flat-topped	rounded	rounded	rounded	rounded	rounded	rounded
Bud shape	ovoid	ovoid	globose	cylindrical	cylindrical	cylindrical	cylindrical	cylindrical
Bud length	8–10 cm	ca. 8 cm	4–7 cm	10–15 cm	24–29 cm	12–18 cm	15–22 cm	ca. 20 cm
Flower orientation	erect	erect	pendent	erect	± horizontal	± erect	± horizontal	± erect
Calyx color—outside	brown	green	green	green	green	green	green	green
Calyx color—inside	cream	cream	cream	cream	red	red	red	red or cream
Calyx tube shape	cup-shaped	cup-shaped	flat	cylinder	cylinder	cylinder	cylinder	cylinder
Calyx length vs. corolla	longer	longer	longer	shorter	longer	longer	longer	longer
Annular nectar chamber	absent	absent	absent	weak	weak	distinct	distinct	weak
Petal color	white	white	white	white	yellow	dark red	yellow	yellow
Petal shape	linear	linear	obovoid	oblanceolate	linear	linear	linear	oblanceolate
Petal widest point	above mid.	above mid.	above mid.	above mid.	below mid.	below mid.	below mid.	above mid.
Petals vs. androecium	longer	± equal	longer	longer	shorter	longer	longer	longer
Petal length	9–10 cm	8–10 cm	8–12 cm	10–15 cm	12–16 cm	12–18 cm	14–20 cm	15–24 cm
Petal length : width	ca. 5 : 1	ca. 5 : 1	ca. 1 : 1	ca. 5 : 1	>10 : 1	>10 : 1	>10 : 1	ca. 5 : 1
Staminal tube length	8–10 mm	9–12 mm	30–50 mm	25–50 mm	60–90 mm	50–90 mm	50–80 mm	130–190 mm
Free filament length	35–65 mm	50–75 mm	30–50 mm	20–80 mm	70–140 mm	50–110 mm	50–120 mm	10–20 mm
Central bundle present	no	no	no	no	yes	no	no	no
Free filament number	600–700	800–1100	720–1600	150–350	120–160	90–100	100–150	200–250
Anther size	large	large	large	small	small	small	small	small
Style color	white	white	white	white	red	red	red	pink/red

TABLE 2. Continued.

Character	Section *Brevitubae*		Section *Adansonia*	Section *Longitubae*				
	A. grandidieri	*A. suarezensis*	*A. digitata*	*A. gibbosa*	*A. rubrostipa*	*A. madagascariensis*	*A. za*	*A. perrieri*
Style shape	± straight	± straight	bent	straight	straight	± straight	straight	± straight
Stigma color	pale pink	white	white	white	red	red	red	red
Ovule number	ca. 300	?	?	350–600	ca. 300	?	?	?
Phenology								
Flowering season	June–Aug.	May–June	Nov.–Dec.	Nov.–Jan.	Feb.–Mar.	Mar.–Apr.	Nov.–Jan.	Nov.–Dec.
Flowering when in leaf	no	no	usually	usually	yes	yes	yes	young leaves
Peak number of flowers/night	40–80	30–50	10–50	10–40	10–20	20–30	20–30	10–20
Morphological Development								
Time of anthesis	1750–1820	1630–1745	1930–2000	1930–2130	1915–2115	1730–1900	1830–1945	1730–1900
Time to open (min.)	10–20	20–60	10–20	20–30	0.5–2	5–10	1–3	15–30
Flower persistence	2.5–4 days	0.5–3 days	1 day	2–4 days	1.5–3 days	3–4 days	1 day	2–3 days
Style persistent	yes	yes	yes	yes	no	no	yes	no
Calyx persistent	yes	yes	yes	yes	no	no	no	usually
Nectar								
Maximum volume (μl)	1400–1730	ca. 1900	?	64–180	59–105	250–620	200–250	150–200
Concentration (%)	13.5–18.25	11.75–12	?	17–23.5	13–15.75	?	21–22	14.5–15.25
Sucrose : hexose (n)	?	?	0.53–1.38 (4)	?	?	0.77–1.92 (4)	0.36–1.13 (12)	?
Scent								
Description	sour	sour	sour/musky	sweet	sweet	sweet	sweet	sweet

surface and on the petals. In section *Brevitubae* the nectar accumulates in an open, cuplike depression up to 1.5 cm deep. In section *Longitubae* the calyx tube fits tightly around the bases of the petals, restricting access to the nectar. In two species of *Longitubae*, *A. za* and *A. madagascariensis*, this calyx tube may have a distinct annular nectar chamber (Fig. 2G).

The corolla of *Adansonia* is composed of 5 (rarely 4 or 6) free petals attached to the base of the androecium. The petals vary in shape (see Table 2) and usually overlap for part of their length. Section *Brevitubae* tends to have relatively narrow (but sturdy) petal bases with large gaps in between, providing easy access to the nectar. In *Longitubae*, there are smaller gaps and a greater degree of overlap, hence it is more difficult to extract nectar. However, the lower parts of the petals overlap in a convolute pattern forming a cone below. The smooth inner surface of the petals would, thus, direct a flexible proboscis to one of the five angled entry points of the nectar chamber, one between each pair of petals.

In section *Brevitubae* the petals are reflexed, clasping the calyx, and are almost obscured by the spreading androecium (Fig. 2A, B). In contrast, *A. digitata* (sect. *Adansonia*) has very broad petals, which play an important role in visual display (Fig. 2C). The upper (abaxial) surface of the petals accumulates drops of nectar dislodged from the calyx. In section *Longitubae* the petals also play an important role in visual display. The flowers of *A. gibbosa* are white, becoming cream or yellow with senescence (Fig. 2D), while in *A. rubrostipa*, *A. za*, *A. perrieri*, and some *A. madagascariensis* the petals are yellow (Fig. 2E, G, H), often with a diffuse reddish streak on the adaxial surface in *A. za*. Most populations of *A. madagascariensis* have dark red petals, which provide a striking contrast to the pale androecium (Fig. 2F).

The androecium comprises a staminal tube surmounted by numerous free filaments (I will refer to the free portions of the androecium as "filaments" while recognizing that the staminal tube is probably also derived from filament tissue). In all species of *Adansonia* the androecium is white or pale yellow (tending to darken with age), and more or less glabrous. There is considerable variation in androecial form in the genus. As the name of the section suggests, *Brevitubae* have very short staminal tubes that do not significantly exceed the top of the ovary and whose width exceeds their length. Sections *Adansonia* and *Longitubae* have tubes at least twice as long as the ovary. In section *Longitubae*, except *A. perrieri*, the tubes are significantly shorter than the filaments, whereas in *A. digitata* (sect. *Adansonia*) and *A. perrieri* (sect. *Longitubae*) they slightly or greatly (respectively) exceed the length of the filaments (see Table 2, Fig. 2H).

The number of filaments is significantly higher in sections *Brevitubae* and *Adansonia* than in *Longitubae* (Table 2). Since the anthers of *Longitubae* are smaller, and there is no clear difference in ovule number among the sections, sections *Brevitubae* and *Adansonia* presumably have a higher pollen : ovule ratio.

The positioning and length of the filaments affect the distribution of the anthers. In *Brevitubae* the outer filaments are slightly longer than the inner and they spread horizontally over the top of the calyx cup (Fig. 2A, B). In *A. digitata* (sect. *Adansonia*), the relatively short filaments spread out from the top of the staminal tube forming a complete sphere, ovoid, or disc (Fig. 2C). In *Longitubae* (except *A. perrieri*) the long, free filaments form a funnel; however, there are modifications of this basic structure. In *A. gibbosa* the inner filaments are shorter than the outer, while the reverse is true of *A. rubrostipa*, *A. madagascariensis*, and *A. za*. *Adansonia rubrostipa* is unique in the genus in having a secondary staminal tube. This "inner bundle" comprises 10–15 central filaments that are fused for about 8 cm above the top of the primary staminal tube (Figs. 12, 14). This inner bundle concentrates the anthers at the center of the flower around the style. The androecium of *A. perrieri* is unique in *Longitubae*, comprising a long, slender staminal tube surmounted by short filaments, which spread out in all directions, as in *A. digitata* (Fig. 2H).

The anthers have a single, long, sinuous pollen sac running around the edge of the connective. In sections *Brevitubae* and *Adansonia* the insertion of the filament is subbasal and non-versatile, whereas in *Longitubae* it is more or less central and somewhat versatile.

The gynoecium consists of a syncarpous ovary and a single terminal style. The ovary and the lower region of the style have a dense indumentum of sharp, upward-pointing hairs up to 2 mm long. The shape of the ovary varies slightly, showing a general correlation with the shape of the mature fruit, but this does not appear to be of any significance for pollination. In contrast, style color, length, and shape are potentially important.

The style of sections *Brevitubae* and *Adansonia* and *A. gibbosa* in section *Longitubae* is white (Fig. 2A–D), while that of *A. rubrostipa*, *A. madagascariensis*, *A. za*, and *A. perrieri* is red or pink

(Fig. 2E–H). The styles of *A. grandidieri* (sect. *Brevitubae*) and *A. madagascariensis* (sect. *Longitubae*) are sometimes longer than the calyx and corolla and then they are slightly bent in the bud. More extreme is *A. digitata* (sect. *Adansonia*) with a long style that is usually bent over at approximately right angles in the globose bud (Fig. 2C). Baker (1985) suggested that the bent style of *A. digitata* might be an adaptation for bringing the stigma close to the base of the flower where it is more likely to be touched by a visiting bat. However, the possibility that this character is a developmental by-product of constraining a long style inside a bud needs to be considered. The styles of section *Brevitubae* and section *Adansonia* are sturdier than those in section *Longitubae*.

In sections *Adansonia* and *Longitubae* the stigmas, when fully open, are composed of 5–10 lobes. In *A. digitata*, and to some extent *A. gibbosa* and *A. perrieri*, the lobes are well defined whereas in *A. rubrostipa*, *A. za*, and *A. madagascariensis* they are irregular and poorly defined. In section *Brevitubae*, *A. suarezensis* has a yellowish, club-shaped stigma with no discernible lobes, whereas *A. grandidieri* has a small, irregular, pinkish stigma.

(2) Phenology. *Adansonia digitata* usually flowers at the start of the wet season (the timing of which varies across Africa), but there are many populations that flower at other times of the year (Wickens, 1983). Those introduced into Madagascar flower in November, the beginning of the wet season. Both species of section *Brevitubae* flower during the dry season (May to September). All section *Longitubae* flower during the wet season (November to March): *A. gibbosa*, *A. za*, and *A. perrieri* at the beginning of the season (November to January); *A. rubrostipa* in the middle (February to early March); and *A. madagascariensis* at the end (March to April). It is noticeable that all known Malagasy populations comprising more than one baobab species have staggered flowering with no two species overlapping. This pattern suggests character-displacement (e.g., Gentry, 1974), but such a hypothesis is difficult to evaluate.

In all the species examined, flowering phenology fits a modified steady-state pattern (Gentry, 1974; Hopkins, 1984). Flowering extends over approximately four to six weeks with relatively few flowers per night. Sections *Adansonia* and *Longitubae* appeared to produce fewer flowers per night (1–30/tree) than *Brevitubae* (30–80/tree), but further work in other localities would be useful to confirm this.

For *A. rubrostipa* (sect. *Longitubae*), the mean number of flowers open on a given night during the peak of the flowering season was 0.88 per tree. Of those trees producing flowers on a given night, the mean was 2.96 flowers. Only 5 of 25 trees averaged more than one flower per night for the 15 days on which observations were made. The highest number of flowers opening on one night on a single tree was 27, and this same individual also had the highest mean number of flowers (4.81/night). Four of the 25 trees produced no flowers during the 15 days of observation and 13 produced 5 or fewer.

(3) Floral development. *Adansonia* flowers develop in the axils of leaves or bracts, usually at the tips of branches. The very young buds of all the species studied in sections *Longitubae* and *Brevitubae* are ovoid. Buds in section *Brevitubae* retain this shape, while those of section *Longitubae* grow in length more than width, becoming elongated and cylindrical when mature. The bud growth of *A. digitata* (sect. *Adansonia*) was not examined.

On the day of anthesis, floral buds grow quickly (as much as 4 mm/hr. in *A. rubrostipa*) and generally become paler. In all species studied, pollen is released in the bud approximately 2–6 hours before anthesis. A minimal amount of nectar also accumulates.

Anthesis, here defined as the opening of the flower bud from the earliest splitting of the calyx until the lobes are fully free and twisted at the base of the flower, takes place in the evening in all species of *Adansonia*. In *A. suarezensis* (*Brevitubae*) and *A. madagascariensis* (*Longitubae*) anthesis commences before dark, as early as 1630h in the former case (sunset at approximately 1730h) and 1730h in the latter (sunset at approximately 1800h). The other species all undergo anthesis after dark, usually within an hour of dusk (up to 2.5 hr. in *A. rubrostipa*).

The flowers of a tree are generally well synchronized, all opening within an hour of each other. The synchronization between trees is weaker, with opening spread out over as much as 2.5 hr. In the case of *A. rubrostipa*, individual trees could generally be characterized as either late or early openers. It is not known whether intrinsic (circadian) or external cues determine the time of anthesis.

Adansonia species fall into two groups with respect to the rate anthesis (Table 2). In *A. grandidieri*, *A. suarezensis*, *A. digitata*, *A. gibbosa*, and *A. perrieri* anthesis takes from 10 to 60 minutes, the slowest being the day-opening *A. suarezensis*. In *A. rubrostipa*, *A. madagascariensis*,

and *A. za* anthesis is spectacularly rapid, being easily followed with the naked eye. The buds (15 to 29 cm long) usually take only 2–3 minutes to open, but sometimes as little as 30 seconds in *A. rubrostipa*. The cellular basis of the rapid anthesis is unknown. However, the outer layer of the calyx is rigid and growth or cell expansion in the inner layer could be responsible for the movements of the lobes during anthesis.

At the end of anthesis (as defined here), the calyx is loosely coiled at the base of the flower and the petals and androecium are partially expanded. During the next few hours the coiling of the calyx becomes progressively tighter and the filaments gradually spread outwards. However, this intensified turgor lasts only about 6 hours, after which the floral parts become more flaccid.

Three of the species studied (*A. suarezensis*, *A. digitata*, and *A. za*) had flowers that usually abscised within 24 hours of anthesis. In all three, the styles persist after the corolla and androecium have abscised (presumably to allow for the completion of pollen-tube growth). The other species had flowers lasting two to four days and of these *A. rubrostipa*, *A. madagascariensis*, and *A. perrieri* have caducous styles that fall attached to the androecium.

The calyx is persistent in section *Brevitubae*, *A. digitata* and *A. gibbosa*, whereas in *A. rubrostipa*, *A. madagascariensis*, and *A. za* it is caducous with a well-defined abscission zone close to its base. *Adansonia perrieri* is variable, in that the calyx is usually caducous but sometimes somewhat persistent.

(4) Scent. In sections *Brevitubae* and *Adansonia* the flowers have a sour, none-too-pleasant smell. The closest description I can give of the odor of *A. grandidieri* is "sour watermelon." Flowers of section *Longitubae*, in contrast, have a sweet, pleasant fragrance. In *A. gibbosa* the scent is heavy and reminiscent of vanilla. In the others it is lighter and more gardenia-like.

(5) Nectar. Comparative nectar data are presented in Table 2. Quantitative measurements of nectar volume were not made on *A. digitata*, but I observed large droplets of nectar on the inner surface of the calyx and estimate that at least 500 μl is produced by this species. Hence, it appears that the total volume of nectar produced by sections *Adansonia* and *Brevitubae* is much higher than in section *Longitubae*.

The course of nectar production was studied in more detail in *A. grandidieri*, *A. gibbosa*, *A. za*, *A. perrieri*, and *A. rubrostipa* (Figs. 3, 4).

In *A. grandidieri*, nectar production started at anthesis and continued at a fairly constant rate (approximately 110 μl/hr.) throughout the night, slowing around dawn (52–75 μl/hr.). It was not determined when nectar flow ceased. Despite some variability, it appears that *A. za* (Fig. 4A) and *A. gibbosa* (Fig. 3C) produce nectar constantly throughout the night, but at a much slower rate than *A. grandidieri* (Fig. 3A; note the scale of the y-axis). Two of the *A. gibbosa* trees studied and the single *A. za* appeared to show nectar resorption soon after dawn, but the effects of sampling error cannot be ruled out.

Although sparse, the data for *A. rubrostipa* suggested that most of the nectar is produced before and soon after anthesis (Fig. 3E), whereas in *A. perrieri* it occurs between 2100h and 0300h (Fig. 4C). However, the low nectar production by *A. rubrostipa* during the night could be an artifact caused by progressive damage to the nectariferous tissue during nectar extraction.

Nectar concentration data are summarized in Table 2. The nectar concentration of section *Brevitubae* (ranging from 11.75 to 18.25%) is within the range of known bat-pollinated taxa (e.g., 11.75 to 15.4%, Ramirez et al., 1984; 26.6 ± 1.5% and 27.2 ± 1.9%, Kress, 1985). In *Longitubae*, *A. za* (19 to 22%) and the Australian species *A. gibbosa* have a nectar concentration similar to the average of 22.1% for hawkmoth-pollinated plants reported by Pyke & Waser (1981). The other Malagasy *Longitubae* (*A. rubrostipa*, *A. perrieri*, and *A. madagascariensis*), with a range of 13 to 18.5%, have more dilute nectars similar to the mean of 13.3% reported for a Malagasy sphingophilous orchid species (Nilsson et al., 1985, 1987).

Figures 3 and 4 also summarize the changes in the nectar concentration throughout the night for *A. grandidieri*, *A. gibbosa*, *A. rubrostipa*, *A. perrieri*, and *A. za*. Four of the five *A. grandidieri* trees, *A. za*, and *A. perrieri* had nectar that declined slowly in concentration during the night. In *A. rubrostipa* it remained more or less constant in the few hours after anthesis but had decreased by the next morning. Changes in nectar concentration in *A. gibbosa* varied greatly between trees. However, within a tree a fairly consistent pattern was detected in which nectar concentration remained fairly constant during the night but became weaker after dawn.

The analyses of nectar composition are shown in Table 2. Sucrose : hexose ratios were variable within species but tended to be relatively rich in sucrose. *Adansonia digitata* has a mean ratio of

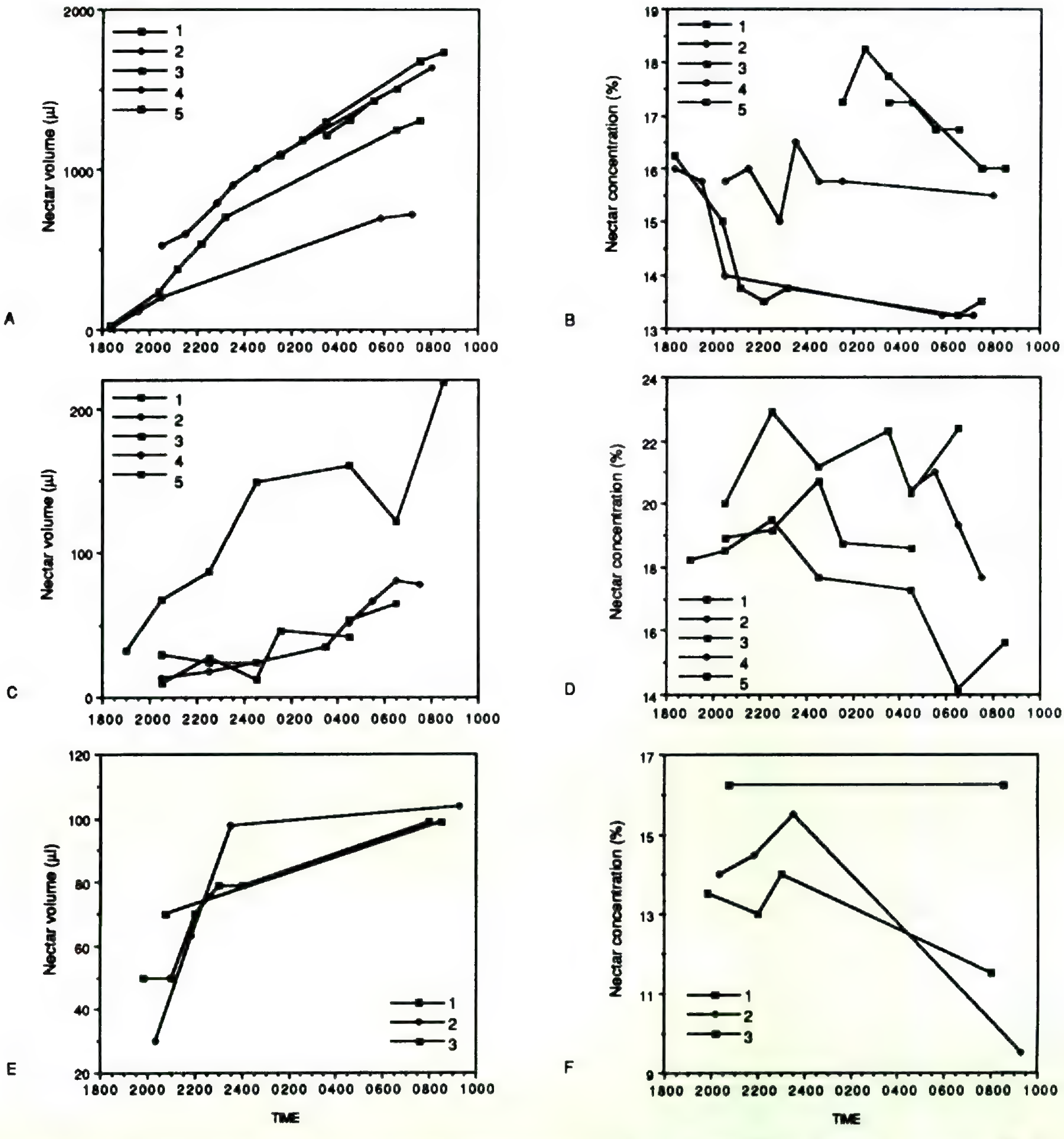


FIGURE 3. Nectar production in *Adansonia grandidieri* (A, B), *A. gibbosa* (C, D), and *A. rubrostipa* (E, F). Figures show cumulative nectar volume when repeated collections from the same flower (E) or actual nectar volume when separate flowers sampled (A, C). Nectar concentration is given in percent sucrose equivalent (B, D, F). Each point on C and D represents an average of 2–8 flowers from the same tree.

0.87, which is more sucrose-rich than for most bat-pollinated plants but not unusual for an Old World species (Baker & Baker, 1983). This value is also typical of the few non-flying-mammal pollinated plants studied (Baker & Baker, 1983).

The nectar of *A. za* had a very variable sucrose : hexose ratio ranging from 0.36 ("hexose-rich") to 1.13 ("sucrose-dominated"). The mean, however, based on 10 samples from throughout the range, was 0.68 ("sucrose-rich"; Baker & Baker, 1983). In *A. madagascariensis*, the nectar had the highest sucrose : hexose ratio of the three species studied, and with a mean of 1.38, it is sucrose-dominated. Sucrose-dominated nectar is particularly common in hawkmoth flowers, though it is also known from paleotropical bat flowers and non-flying-mammal flowers (Baker & Baker, 1983).

Overall, the sucrose : hexose ratios of *Adansonia* show no clear pattern of interspecific variation, possibly because both Old World mammals and hawkmoths (the two major pollinators, see below) favor sucrose-rich nectar.

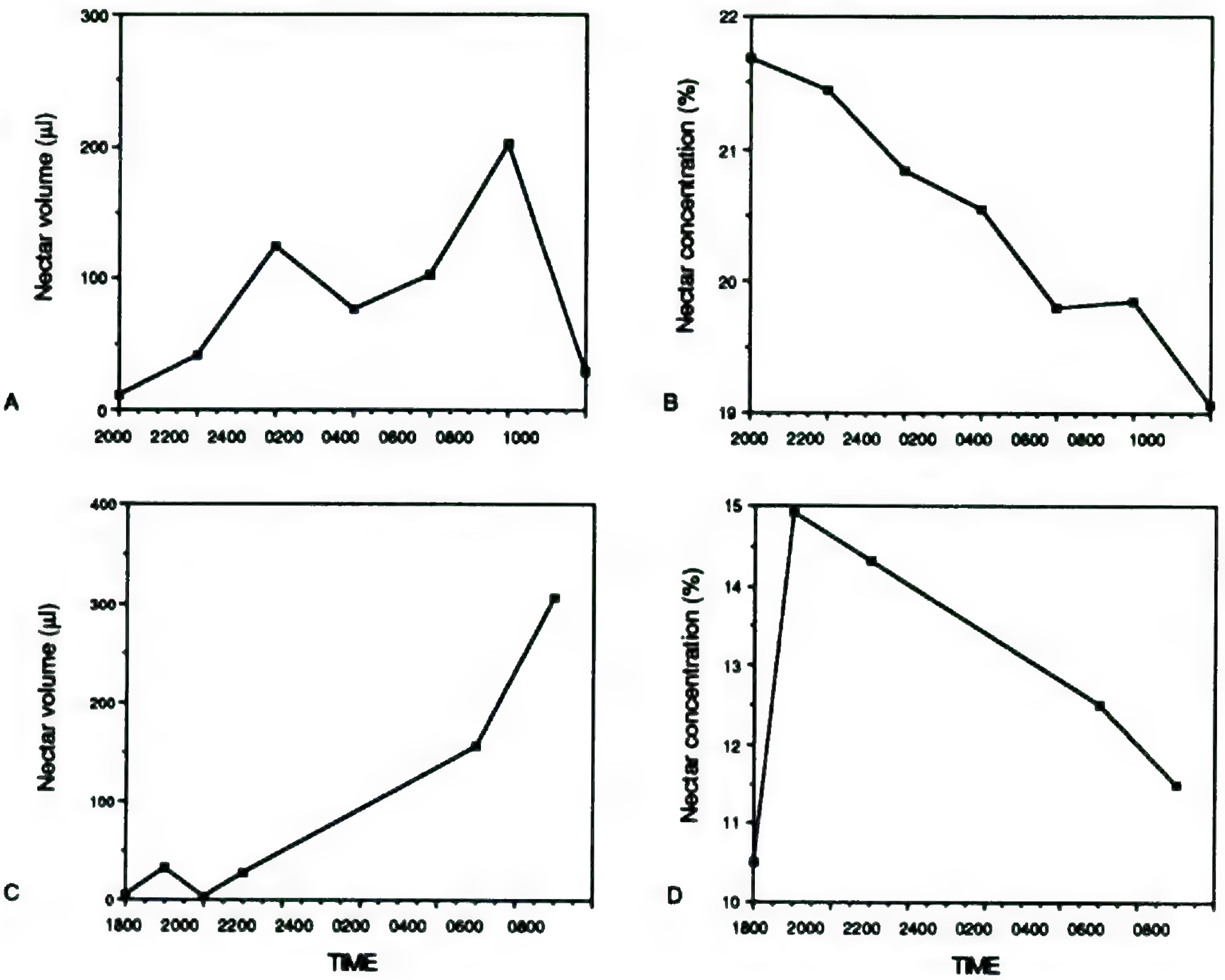


FIGURE 4. Nectar production in *Adansonia za* (A, B) and *A. perrieri* (C, D). Figures show actual nectar volume (A, C) or nectar concentration (percent sucrose equivalents; B, D) plotted against time. Each point represents an average of 2–5 flowers from the same tree.

In *A. za*, relatively high concentrations of amino acids (8–10 on the histidine scale) were detected (I. Baker, unpublished data). In two samples analyzed further, glutamine and asparagine were the most abundant, followed closely by serine (I. Baker, pers. comm.). The significance of these data is unclear.

(6) Stigmatic receptivity. In species with well-developed stigmatic lobes, the lobes start to expand either before or just after anthesis, having been infolded in bud. In all *Adansonia*, brown, senescent tissue appeared on the stigma by dawn or soon after. By mid-morning the stigmas are completely brown and dry (in the absence of rain). In *A. gibbosa*, the stigmatic lobes, as well as browning, often become slimy and wet between about 0200h and 0400h, drying out again later.

The peroxidase test was positive in all species either at anthesis or within an hour afterwards. As an indicator of general metabolic activity these data are consistent, with stigmatic receptivity commencing about the time of anthesis. This is further supported by the breeding system experiments (reported below) in which pollen was applied to flowers at or soon after anthesis. In all cases, these hand pollinations successfully led to pollen-tube growth, and in the case of *A. gibbosa* fruit set was also observed.

In *A. gibbosa* a hand-pollination experiment was conducted in order to determine whether stigmas remain receptive in the early morning. Of the 19 flowers pollinated during the night (13 at 2200h, 6 at 2400h), 6 (31.6%) remained attached one month after pollination, indicating successful fertilization. Of the 31 pollinated in the early morning (13 at 0400h, 6 at 0500h, 13 at 0600h), 15 (48.4%) were still attached one month later. Hence, *A. gibbosa* stigmas remain receptive into the early morning.

(7) Breeding systems. Examination of all open- or hand-pollinated styles under the microscope revealed characteristic spinulose pollen grains on the stigma. Pollen tubes were visible just below the stigma, but they were less apparent further down the style, perhaps due to differences in callose deposition (aniline blue stains callose). A few iso-

TABLE 3. Breeding experiment on *A. gibbosa*. The four trees were at the Oscar Range site. Pollinations were carried out between 2030 h and 2130 h on 24–30 Nov. 1989. The number of fruits still attached to the tree on 1 Jan. 1990 is shown. The number of flowers in each category is given in parentheses.

Tree	Number of days fruit allowed to develop	Distance to pollen donor	Number remaining (Number at start) Unpollinated	Self	Cross
1	36	4.5 km	0 (10)	0 (10)	10 (10)
2	38	350 m	–	1 (10)	6 (10)
3	34	115 m	0 (5)	0 (4)	2 (5)
	32	115 m	0 (5)	0 (6)	4 (5)
4	34	8 m	0 (5)	0 (4)	4 (5)
	32	8 m	1 (5)	0 (6)	4 (5)
Totals			1 (30)	1 (40)	30 (40)

lated pollen tubes with irregular callose plugs could often be seen, however. At the very base of the style, where the conducting tissue narrows just before entry into the ovary, the pollen tubes were highly visible and often very numerous. Thus, while the poor visibility for much of the style limited the accuracy with which pollen-tube growth can be measured, the high visibility at the base of the style permitted it to be determined with confidence whether pollen tubes had successfully penetrated the ovary.

In *A. rubrostipa* and *A. madagascariensis* pollen tubes were given from 18 hr. 10 min. to 42 hr. 30 min. to grow, but none reached the bottom of the style during that time. Despite the few styles examined (five for *A. rubrostipa*, six for *A. madagascariensis*), there was no evidence that the growth of self-pollen-tubes was any less than that of cross-pollen-tubes; in fact, the self- had, on average, penetrated further than the cross-pollen-tubes. In the cases of *A. grandidieri* and *A. gibbosa*, both self- and cross-pollen-tubes grew down the entire style with no noticeable difference in rate. Of the 28 styles of *A. grandidieri* (13 selfs, 15 crosses), only one lacked pollen tubes at the base. Of the 10 styles of *A. gibbosa* (7 selfs, 3 crosses), only two lacked pollen tubes at the base. A single self-pollinated style of *A. suarezensis* was likewise observed to have pollen tubes at the base.

These data suggest that there is no inhibition of self-pollen-tube growth in the style. Furthermore, in *A. gibbosa* both cross- and self-pollen-tubes were observed entering the ovules through the micropyle. Pollen-tube growth data thus suggest self-compatibility in the *Adansonia* species studied.

In order to investigate whether the equality of pollen-tube growth resulted in equal fruit set of selfs and crosses, a hand-pollination experiment was carried out in *A. gibbosa*. Almost all the unpollinated and selfed flowers had aborted 32–38 days after anthesis (Table 3). In contrast, 75% of the cross-pollinated flowers remained attached to trees at that time. This suggests that the flowers are functionally self-incompatible. Since the pollen-tube data show that self-pollen-tubes can successfully penetrate the ovules, incompatibility must be late-acting (Cope, 1962; Seavey & Bawa, 1986). Late-acting self-incompatibility involving early abortion of fertilized ovules is known in *Chorisia* (Gibbs & Bianchi, 1993) and *Eriotheca* (Oliveira et al., 1992) also in the Bombacaceae.

POLLINATION BIOLOGY

The results of nocturnal and diurnal floral watches for each species are described below and summarized in Table 4.

(1) Adansonia grandidieri. When observations were made in forested sites near Marofandelia, fork-marked lemurs (*Phaner furcifer* Blainville) made non-destructive visits to the flowers (Fig. 5). One or a pair of animals moved through the canopies of two adjacent baobabs visiting flowers (approx. 2 visits/flower/hr.). The animals inserted their snouts into the sides of the flowers and licked nectar from the petal bases. This behavior results

TABLE 4. The peak visitation rate of classes of floral visitors to *Adansonia* species (abbreviated to first three letters). – = no visits observed. + = 0–1 visits/fl/hr. ++ = 1–10 visits/fl/hr. +++ = >10 visits/fl/hr. Inferred major pollinators are marked with square brackets, minor or possible pollinators with parentheses.

	gra	sua	dig	gib	rub	mad	za	per
Bees, flies, butterflies	++	+++	++	+	+	(+++)	++	+
Settling moths	–	+	+	++	+++	+	+	+
Hawkmoths	++	++	+	[++]	[+++]	–	[+++]	[+++]
Birds	++	++	–	(++)	+	–	++	+
Non-flying mammals	[++]	–	(+)	–	++	–	(++)	–
Bats	–	[+]	[++]	–	–	–	–	–

FIGURES 5, 6. Floral visitors of *Adansonia grandidieri*. —5. Lemur (*Phaner furcifer*) as shown in the photograph (5A) and line drawing (5B). Note pollen deposited on the animal's face. —6. Hawkmoth (*Nephele comma*).

in pollen being deposited on the animal's face (Fig. 5). Although I was not able to see stigmatic contact, in view of the animals' large body size some pollination is likely to occur. *Phaner* is omnivorous, feeding on insects, fruits, flower-buds, flowers, and especially gums (Petter et al., 1975; Charles-Dominique & Petter, 1980). Sussman & Raven (1978) suggested a role for *Phaner* in the pollination of Malagasy plants, but this prediction has not previously been confirmed. Of the other lemur species that are sympatric with *A. grandidieri*, only one other is a confirmed nectar feeder, the dwarf lemur, *Cheirogaleus medius* E. Geoffroy (see below). However, *Cheirogaleus* hibernates during the dry season and is, therefore, not a potential pollinator of *A. grandidieri*.

Despite indications by local people that bats visit flowering *A. grandidieri* trees, no bat visits were observed during two weeks of nocturnal observations. This absence could be due to the proximity of the study site to a village and the fact that fruit bats are trapped locally for food. Additional work in some more isolated populations (e.g., near Lac Ihotry, south of Morombe) is needed to determine whether bats are pollinators of *A. grandidieri*.

In the morning after anthesis a few honeybees and small sweat bees collected pollen, but did not contact the stigmas. Since no effective pollinators visit the flowers after dawn, the bees probably have no detrimental effect and represent commensals.

Hawkmoths identified from photographs as *Nephele comma* Hopfer (L. A. Nilsson, pers. comm.) were reliable visitors in the first 30 minutes after anthesis and the 30 minutes before dawn (Fig. 6). The peak visitation rate observed was 8 visits in 20 minutes (24 visits/flower/hr.), each visit lasting 5–20 seconds. The moths approached the flowers from the side, below the level of the androecium, and thus no pollination occurred. By removing nectar that could attract legitimate pollinators, hawkmoths probably have a slight negative effect on reproductive output.

Soon after dawn, large numbers of sunbirds visited the flowers and fed on nectar and occasionally on small bees that were collecting pollen. *Nectarinia souimanga* Gmelin and *N. notata* Müller were observed with equal frequency. They perched below the flowers on the flower stalk or calyx and fed by inserting their beaks under the filaments (Fig. 7). As a result, despite the high visitation rate (5–10 visits/flower/hr.), they are responsible for little pollen transfer. In the absence of information about the flower's ability to reabsorb excess nectar, it is unclear whether the sunbirds have a net negative or neutral effect on the plant.

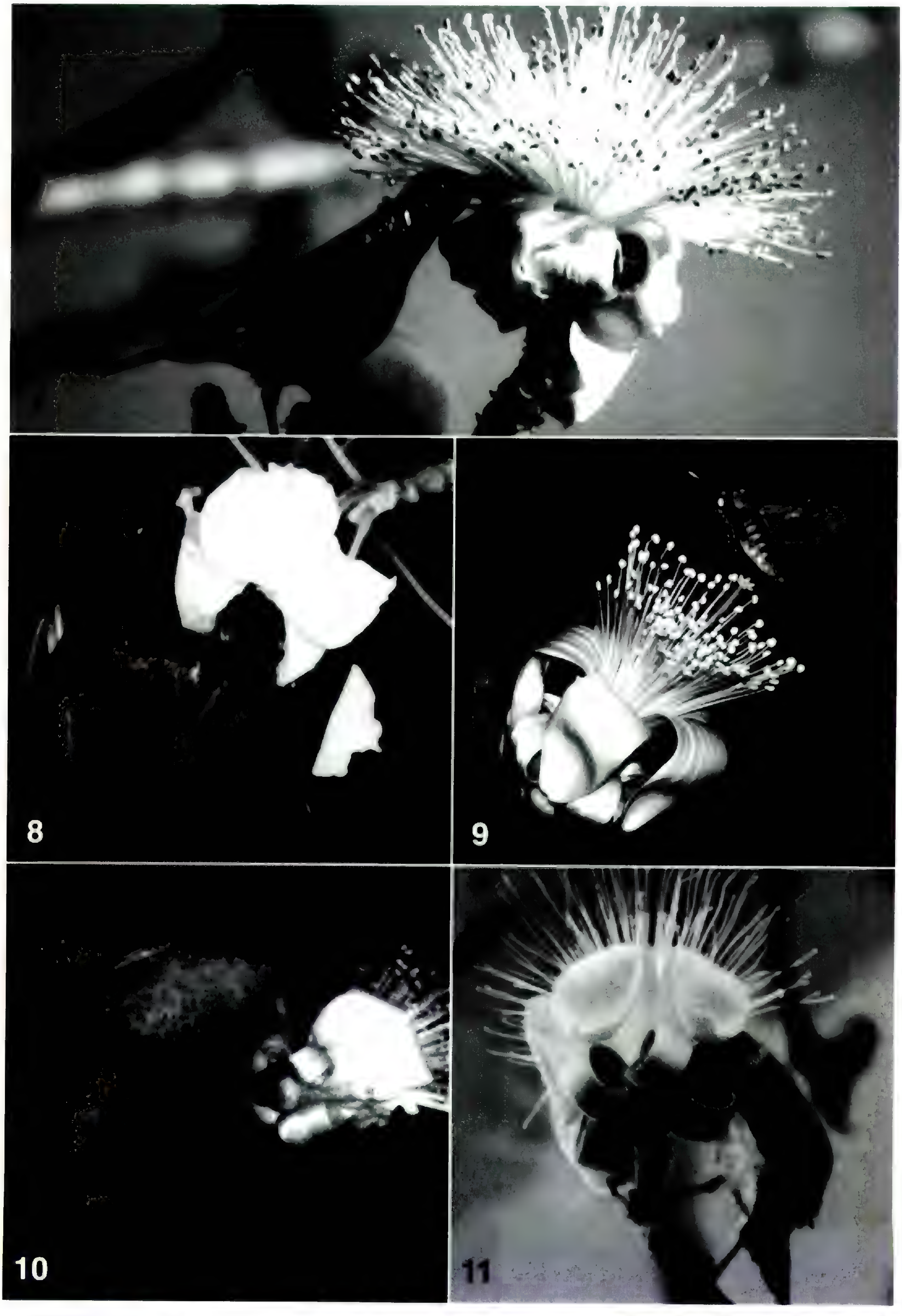
Adansonia grandidieri is clearly pollinated primarily by nocturnal mammals, but further work is needed to determine whether bats, as well as lemurs, play a role. Other floral visitors seem not to contribute significantly to pollination.

(2) Adansonia suarezensis. The first of the two study sites, the Montagne des Français on the west of the Baie d'Antsiranana, is a heavily disturbed patch of deciduous forest merging into overgrazed *Cryptostegia* (Asclepiadaceae) scrub-land. No pollinating visits were observed during more than one week of nocturnal observation. However, at the second study site, the less disturbed deciduous forest at Beantely, visits by fruit bats (probably *Eidolon dupraenum* Pollen) were observed. The visits were concentrated in the hour after dusk, but occurred at a low rate throughout the night (observations made close to full moon). Accurate visitation rates were hard to assess because my presence disturbed the bats. Fourteen visits/tree/hour (approximately 0.5 visits/flower/hr.) was the peak rate observed. The bats landed close to a flower and then clambered over to it. Each visit lasted 20–30 seconds, with as many as nine flowers being visited in succession. Usually, a bat would leave the tree immediately after completing a visit and fly to another flower on the same or another tree, but sometimes it clambered directly from one flower to another.

In view of the bats' large body size and the way they envelop the flowers when feeding, pollen transfer seems inevitable. In view of their strong flight (Heithaus et al., 1974; Faegri & van der Pijl, 1979), fruit bats are likely to cause both self- and cross-pollination. Only three species of fruit bat occur in Madagascar, *Eidolon dupraenum*, *Pteropus rufus* E. Geoffroy, and *Rousettus madagascariensis* G. Grandidier. The latter is restricted to a small area on the east coast, whereas the others occur in coastal areas throughout the island (Dorst, 1947; Sussman & Raven, 1978).

The mature buds of *A. suarezensis* start to open in the late afternoon, as much as one hour before dusk. Numerous honeybees visit the flowers between anthesis and dusk. Honeybees and sweat bees also forage on flowers early in the morning. In all cases pollen rather than nectar was collected and no contact was made with stigmas. Since the bees visit in the evening before the legitimate pollinators, they are probably detrimental to the plants' reproductive output and, thus, represent floral parasites.

During the night, visits by unidentified hawkmoths were occasionally observed. The proboscides



FIGURES 7–11. Floral visitors of *Adansonia grandidieri* (7), *A. digitata* (8), and *A. gibbosa* (9–11).—7. Sunbird (*Nectarinia notata*).—8. Fruit bat (*Roussettus aegyptiacus*).—9. Hawkmoth (*Agrius convolvuli*).—10. Little friarbird (*Philemon citreogularis*).—11. Singing honeyeater (*Lichenostomus virescens*).

were less than 5 cm in length, and the approach was exclusively from below the flower in the same manner as in Figure 6. Thus, these visits did not result in pollination.

Soon after dawn, souimanga sunbirds (*Nectarinia souimanga*), visited the flowers that had opened the evening before. Their behavior was the same as that of the sunbirds that visited *A. grandidieri* (Fig. 7); they perched on the flower stalk or calyx and inserted their beaks underneath the filaments into the cup-shaped nectar chamber. As many as five visits per flower in the first hour after dawn were observed, each lasting from 5 to 60 seconds. During longer visits the birds removed and reinserted their beaks several times from different positions around the periphery of the flower. Despite the regularity of the visits, the sunbirds did not contribute to pollination because of the great distance between the nectar chamber and the stigma. Sunbirds are thus acting as nectar thieves, but since their visits occur after those of the legitimate pollinators and do not visibly make contact with the gynoecium, they will only have a detrimental effect on baobab fitness if the plants can reabsorb excess nectar.

My observations suggest that *A. suarezensis* is primarily pollinated by fruit bats. Although I observed no visits by lemurs, they cannot be ruled out as secondary pollinators in other forests. Birds and insects, although frequent visitors, do not contribute significantly to pollination.

(3) Adansonia digitata. In view of the existing literature (reviewed in Wickens, 1983; Dobat & Peikart-Holle, 1985), little time was spent working on *A. digitata*. However, some nocturnal observations in Kenya confirmed that fruit bats (*Rousettus aegyptiacus*) were frequent visitors, especially in the first two hours after anthesis. They were observed landing on the pendent flowers and licking nectar from between the petal bases (Fig. 8). The visits lasted five to ten seconds, and contact with the anthers and stigma was clearly observed.

(4) Adansonia gibbosa. Hawkmoth visits were not observed until the beginning of January, by which time most baobabs had finished flowering. The only hawkmoth visitor seen was *Agrius convolvuli* L. (Fig. 9). Visits were concentrated in the first half-hour after anthesis and reached a peak rate of approximately 5 visits/flower/hr. The moths hovered in front of the flowers for 3–5 seconds, inserting their proboscides, which are ca. 9 cm long, through the filaments, down to the base of the flower. In the process, they came into contact with both the anthers and stigma and were clearly efficient pollinators.

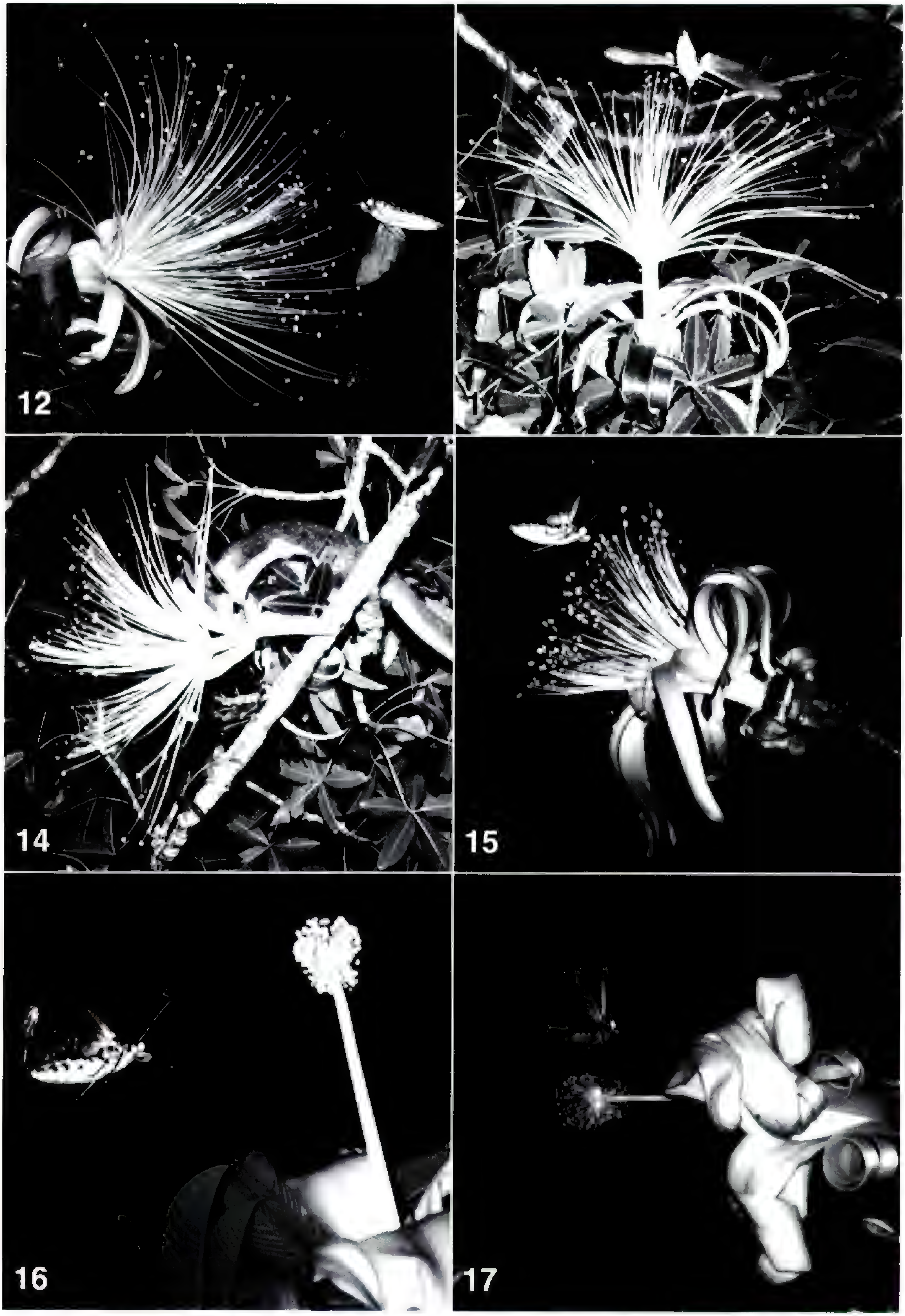
The lack of phenological synchronization between the hawkmoths and baobabs is problematic if hawkmoths are the major pollinators of *A. gibbosa*. Two alternative hypotheses could account for this seasonal discrepancy. Firstly, in highly seasonal climates, lack of synchronization is almost certain to occur occasionally because plants and their pollinators are unlikely to be utilizing exactly the same seasonal cues. Thus, unusual seasons in the study year could, by chance, have resulted in hawkmoth emergence after baobab flowering. Secondly, a hawkmoth species emerging earlier than *Agrius convolvuli* might be the major pollinator of *A. gibbosa*, and this sphingid species might have had a poor season in 1989/1990. Thus, given the lack of any other effective pollinators (see below) and the evidence of self-incompatibility in *A. gibbosa*, hawkmoths are almost certainly the main pollinators, despite the few visits actually observed.

The flowers of *A. gibbosa* were visited by bees collecting pollen in the early morning and by settling moths extracting nectar at night. However, since neither of these flower visitors contacted the stigma, they did not contribute to pollination.

Bat visits were not observed, despite extensive nocturnal observation throughout the range of *A. gibbosa*. However, the blossom bat, *Macroglossus* sp., has been trapped in the vicinity of flowering baobabs (K. Kenneally, pers. com.). Also, van der Pijl (1956) reported claw marks on *A. gibbosa* petals in Java, which he interpreted as being caused by bats. Based on this circumstantial evidence, it is conceivable that bats might occasionally visit the flowers, but they are unlikely to be major pollinators of *A. gibbosa*.

Bird visits were frequent in the early morning (0430–0530h), and occasional at other times of day. Honeyeaters (family Meliphagidae) were the most important (Figs. 10, 11), especially yellow-throated miners (*Manorina flavigula* Gould), little friarbirds (*Philemon citreogularis* Gould), and brown honeyeaters (*Lichmera indistincta* Vig. & Horsf.). Other honeyeaters that visited flowers included: singing honeyeaters (*Lichenostomus virescens* Vieillot), gray-fronted honeyeaters (*Lichenostomus plumulus* Gould), and banded honeyeaters (*Certhyonix pectoralis* Gould), in addition to yellow white-eyes (*Zosterops lutea* Gould) and red-collared lorikeets (*Trichoglossus rubritorquis* Vig. & Horsf.). All these species were primarily nectar feeders. The peak visitation rate was 8 visits/flower/hr., but was usually much lower.

During nectar-foraging the birds perched on the peduncle or calyx, and inserted their beaks into the bases of the flower (Figs. 10, 11). In this position, little if any pollen is applied to the birds



FIGURES 12–17. Floral visitors to *Adansonia rubrostipa* (12–14), *A. za* (15), and *A. perrieri* (16, 17).—12, 13. Hawkmoth (*Coelonia solanii*).—14. Lemur (*Cheirogaleus medius*).—15. Hawkmoth (*Coelonia solanii*).—16. Hawkmoth (*Coelonia solanii*).—17. Hawkmoth (*Xanthopan morgani*).

and no stigmatic contact occurs. In less than 5% of cases birds visited flowers from an adjacent branch, and in this position they could make contact with the stigma. Since flowers are receptive in the early morning (see above) birds might contribute to pollen transfer in *A. gibbosa*, but whether this offsets the detrimental effect of bird foraging (e.g., damage to flowers and/or removal of nectar otherwise destined for reabsorption) remains to be assessed.

(5) Adansonia rubrostipa. The flowers of *A. rubrostipa* were visited throughout the night by the long-tongued hawkmoth, *Coelonia solanii* Boisduval, with a peak of activity soon after anthesis. The visitation rate differed markedly between trees, the highest being 18 visits/flower/hr.

Coelonia approached the flowers from the front, extending their proboscides when about 10 cm away from the flower. The proboscis was inserted through the central filaments (Figs. 12, 13) down to the base of the flower, presumably passing between the petals at their bases and thereby entering the nectar cavity. The moths usually engaged in brief upward and downward movements during the insertion of the proboscis, similar to those described by Brantjes & Bos (1980). However, "swing-hovering" described in *Coelonia solanii* and other Malagasy sphingids (Wasserthal, 1993) was not observed. Visits usually lasted 2–5 seconds, rarely up to 20 seconds, and pollen was scattered on the moths' wings and bodies. Contact with the dark red style and stigma was also observed. Rarely the hawkmoths approached the flowers from the side, inserting their proboscides through the peripheral filaments directly to the flower base. In this orientation they were still dusted with pollen but did not contact the stigma.

Since there were no other sphingophilous plants flowering in Kirindy Forest in February, and since hawkmoths did not spend long in each tree, a high level of inter-tree movement is implicated (see Linhart & Mendenhall, 1977). Thus, *Coelonia* is clearly the major pollinator of *A. rubrostipa*.

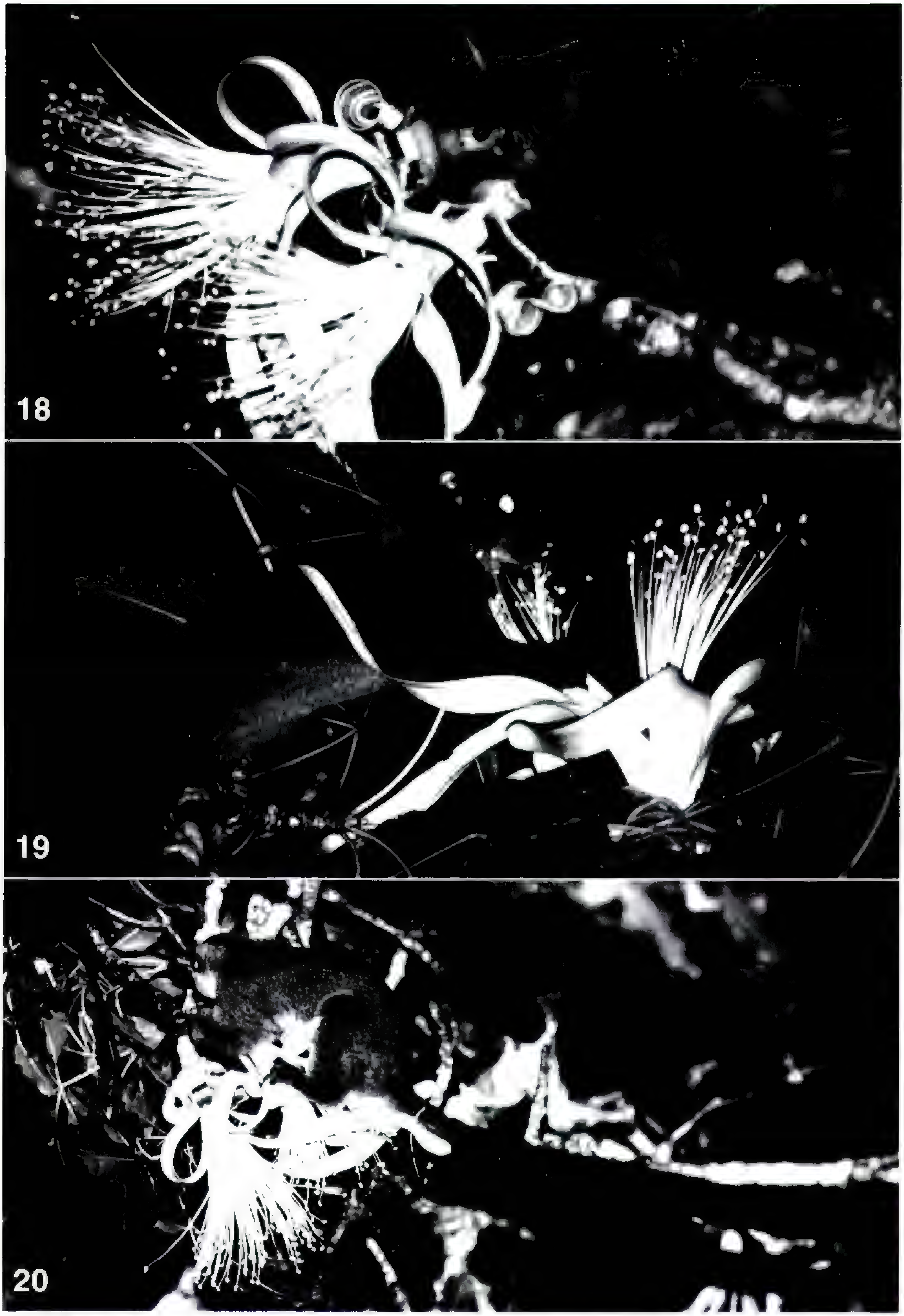
Two species of nocturnal lemur (*Cheirogaleus medius* and *Phaner furcifer*) were frequently observed visiting *A. rubrostipa* flowers. The lemurs collected nectar non-destructively and also hunted insects (mainly settling moths). On a few occasions the lemurs approached the flowers from the apex and, in doing so, rubbed their vents over the anthers and stigma (as depicted for *A. za*, Fig. 20). However, they usually approached from the flower stalk and thus did not deposit pollen on the stigma (Fig. 14). Individual lemurs frequently spent several hours in a single tree, but some movement between trees was observed. Both lemurs are potentially capable of bringing about some pollen transfer, though this might be lower in *Phaner*, which engaged in periodic bouts of grooming and probably removed some of the pollen. Since both species take large amounts of nectar and cause some damage to flowers, their overall effect on reproductive output could be negative.

Diurnal insects such as bees and flies made rare visits in the morning after anthesis but these did not effect pollination. At night, ants and settling moths congregated on the calyx of newly opened flowers (Fig. 2E). In view of the long nectar-to-stigma distance (usually at least 20 cm), these visits did not result in pollination. No damage to floral tissue was observed, but it is unclear whether these animals are nectar thieves (collecting nectar that would otherwise serve as an attractant for legitimate pollinators) or commensals (collecting excess nectar).

In only one case was a Madagascar green sunbird (*Nectarinia notata*) seen visiting a flower, and this was a brief non-pollinating visit lasting 2 seconds.

(6) Adansonia madagascariensis. The study site for the work on *A. madagascariensis*, Montagnes des Français, is heavily disturbed, and this could explain the low frequency of floral visitation observed. The only reliable floral visitors to *A. madagascariensis* were honeybees, which collected pollen from the flowers as they were opening in the late afternoon. As the flowers opened there was a brief period when the spreading calyx lobes formed an open-ended tube extending from just below the stigma down to the anthers. At this time, bees foraging for pollen passed close to the stigma and, thus, bee-pollination was a possibility. However, the brevity of this period (approximately 5 minutes) mitigates against cross-pollination. Further work in less disturbed localities is needed to document the pollination system of *A. madagascariensis*.

(7) Adansonia za. The main pollinators are long-tongued hawkmoths, especially *Coelonia solanii* (Fig. 15). This hawkmoth visited the flowers at a steady rate throughout the night with up to 12 visits/flower/hr. Two other species, *Coelonia brevis* R. & J. and *Panogena jasminii* Boisduval, visited the flowers just after dusk and just before dawn. These species have relatively short proboscides (ca. 10 cm; Wasserthal, 1993), and when visiting flowers, they often approached from the side or lighted on the petals. These behaviors are not well suited to pollination, because contact with



FIGURES 18–20. Lemur (*Phaner furcifer*) visits to *Adansonia za.*—18. A lemur chewing a hole in the annular nectar chamber.—19. A lemur ingesting pollen—20. An example of a visit that could effect pollination.

the stigma is only rarely made. In contrast, *Coelonia* has a longer proboscis (14.6–22.3 cm; Nilsson et al., 1987), which permits it to hover in front of the flower while feeding. During visits, the wings and body of hovering moths made frequent contact with the anthers and stigmas. Furthermore, moths spent only a limited amount of time in a tree, suggesting that they move between trees, thereby permitting cross-pollination.

As well as hawkmoths, fork-marked lemurs (*Phaner furcifer*) were frequent visitors. A group of five individuals visited the same tree on several nights, following one another through the canopy approximately one minute apart. They predominantly fed on nectar, licking the calyx and petal bases (as Fig. 14). They were usually non-destructive, but sometimes chewed open the nectar chamber (Fig. 18), although this did not lead to visible damage to the gynoecium. Some animals were observed to lick the anthers directly (Fig. 19), apparently ingesting pollen. To my knowledge this is the first report of active pollen-feeding in *Phaner*, and the possibility that lemurs digest pollen should be considered (previously reported in nectarivorous marsupials (Turner, 1984) and bats (Howell, 1974)).

As with *A. rubrostipa*, most lemur visits did not result in contact with the stigma. However, a few times an animal approached from another branch, rubbing its front against the anthers and stigma (Fig. 20) and this behavior could lead to pollination. Overall, *Phaner* removes large quantities of both nectar and pollen and deposits relatively small amounts of pollen on stigmas. Furthermore, grooming, which could reduce the amount of pollen carried from tree to tree, was observed. Detailed studies are thus needed to determine whether the net effect of lemurs on the reproductive output of baobabs is positive (due to pollination) or negative (due to pollen/nectar theft). It is clear that even if they have a net positive effect it is less than that provided by hawkmoths.

Pollen collection by bees occurred in the morning. These visits were directed exclusively at the anthers and thus resulted in little or no pollination. Bees are probably commensals, having no negative effect on baobab fitness.

Butterflies occasionally landed at the bases of flowers in the early morning, apparently collecting nectar. They are minor nectar-thieves with either a neutral or negative effect on the trees, depending on the potential for nectar reabsorption.

The large diurnal lemur *Propithecus verreauxi verreauxi* A. Grandidier fed destructively on floral buds and flowers. However, like the other diurnal visitors, they do not contribute to pollination. Thus, it can be concluded that *A. za* is predominantly pollinated by long-tongued hawkmoths with some minor role perhaps being played by nocturnal lemurs.

(8) Adansonia perrieri. The main pollinators of *A. perrieri* are long-tongued sphingids, *Coelonia solanii* (Fig. 16) and *Xanthopan morgani* Walker (Fig. 17), which have bodies of 5–6 cm and proboscides exceeding 20 cm. *Coelonia* is the main pollinator of *A. za* and *A. rubrostipa*, whereas *A. perrieri* is the only baobab I observed being visited by *Xanthopan*. *Xanthopan* is, however, an important pollinator in Madagascar (Nilsson et al., 1985, 1987) and is the subspecies predicted to exist by Darwin (1862).

Most visits by hawkmoths occur just after dusk, with many fewer later in the night. Moths hover in front or slightly to the side of the flowers and insert their long proboscides through the petal bases to reach the nectar (Fig. 17). Each visit lasts 1–2 seconds, but a moth will occasionally visit a flower several times in quick succession. The visitation rate varied greatly from night to night and from tree to tree. The peak observed was approximately one visit/flower/min. for 20 minutes. At times, moth activity was so intense that moths engaged in aggressive interactions in order to gain access to flowers. This intense foraging occurred when there were only limited resources available to hawkmoths due to the almost simultaneous cessation of flowering of several hawkmoth-pollinated plants in the forest (Solanaceae—*Datura* sp., Meliaceae—*Turraea* sp., and an unidentified Amaryllidaceae).

Bees and butterflies were observed visiting flowers in search of pollen and nectar, respectively. However, they were infrequent visitors and did not contribute to pollination because of their lack of contact with stigmas. The only vertebrate flower visitors were sunbirds (*Nectarinia souimanga*), which occasionally visited in the early morning. They drank nectar while perching on the flower stalk and did not contact the anthers or stigmas.

No short-tongued hawkmoths stole nectar, which suggests that they are absent from Montagne d'Ambre in the flowering season. Thus, long-tongued hawkmoths were the only animals observed visiting *A. perrieri* flowers in a manner conducive to pollen-transfer.

Discussion

Looking at the genus as a whole, it is clear that *Adansonia* manifests a considerable diversity in its floral biology, much of which reflects interspecific differences in pollination biology. Now that the

pollination and floral biology of the extant species of baobab is documented (with the exception of *A. madagascariensis*), the next challenge is to elucidate the evolutionary mechanisms that have led to this floral diversity. A number of specific questions need to be asked. To what extent has natural selection acted on individual floral traits in regard to improved pollination by the current pollinating agents? That is to say: what floral traits are adaptations sensu Gould & Vrba (1982)? Likewise, what floral traits are exaptations: traits that have utility under the current pollination system but evolved for some other reason? Furthermore, which phenotypic traits evolved as developmental byproducts of selection acting on other parts of the flower?

As Gould & Lewontin (1979) and Gould & Vrba (1982) pointed out, these questions are intrinsically historical and cannot be answered by looking at current configurations alone. We need some knowledge of the evolutionary history. In the case of *Adansonia* we need to know whether mammal- or hawkmoth-pollination is ancestral and when various floral characters evolved with respect to the switch in pollination system (Greene, 1986; Baum & Larson, 1991). Fossil evidence is unavailable, so the only source of such information is phylogenetic analysis. In the future I hope to evaluate the adaptive status of many floral characters using a phylogenetic approach (see Baum & Larson, 1991). A prerequisite for such a study is a set of clearly defined adaptive hypotheses. In the remainder of this paper I highlight characters that seem to "fit" the physical, behavioral, and sensory attributes of the pollinating animals and propose them as hypotheses of adaptation. I stress that these are just hypotheses. If they appear speculative at times it should be remembered that my purpose is to focus attention on interesting areas for further research rather than to imply that adaptation prevails in shaping the floral biology of *Adansonia*.

SECTION *BREVITUBAE* AND
SECTION *ADANSONIA*

The data for section *Brevitubae* suggest both species are primarily pollinated by mammals. Fruit bats play the major role in *A. suarezensis*, but in the case of *A. grandidieri* nocturnal lemurs are the main pollinators, at least in the vicinity of Marofandelia. Although lemurs have been shown to be important pollinators of some Malagasy plants (Nilsson et al., 1993; Kress et al., 1994) and non-flying mammals have been shown to pollinate some Bombacaceae species in the Neotropics (Prance, 1980; Janson et al., 1981; Steiner, 1981; Gribel, 1988), bat-pollination elsewhere in the range of *A. grandidieri* cannot be ruled out.

Potential nectar and pollen thieves in section *Brevitubae* include bees, sunbirds, hawkmoths, and possibly nocturnal lemurs. Overall, these data suggest a great similarity between the pollination system of sections *Brevitubae* and *Adansonia*. Bats are the major pollinators of *A. digitata*, whereas hawkmoths are thieves, and bushbabies play similar roles to the nocturnal lemurs in Madagascar (i.e., they are either minor pollinators or nectar thieves).

Several characters of sections *Brevitubae* and *Adansonia* seem suited equally to pollination by bats and non-flying mammals. Nocturnal, pale-colored flowers with musky scent and copious nectar are typical of both bat- (Faegri & van der Pijl, 1979) and non-flying-mammal-pollinated plants (Janson et al., 1981; Turner, 1982, 1983; Weins et al., 1983; Rebelo & Breytenbach, 1987). Similarly, the large pollen : ovule ratio (relative to *Longitubae*) has been suggested to be favorable for bat-pollinated plants due to the large surface area of the pollinators (Heithaus et al., 1974), and a similar line of reasoning would apply to non-flying mammals.

Fruit bats are large, not particularly agile flyers and plants pollinated by them frequently have easily accessible flowers (van der Pijl, 1941; Marshall, 1983). In section *Adansonia* this is achieved by penduliflory, whereas in section *Brevitubae* the flowers are borne on erect, sturdy stalks and the crowns have a flat-topped, "pagoda" form that is common in bat-pollinated trees (Marshall, 1983). These alternative "solutions" seem in turn to account for several of the other morphological differences between the flowers of these sections. For example, *A. digitata* has broad petals on which the nectar accumulates, whereas in *A. grandidieri* and *A. suarezensis* it collects in the cup-shaped calyx. Also, the two flower positions can be assumed to affect the behavior of floral visitors. Penduliflory is especially suited to fruit bats, which generally approach from below and land head-up on flowers (Hopkins, 1983, 1984). However, penduliflory must limit accessibility to bushbabies and other non-flying mammals. Erect flowers, on the other hand, might perhaps be handled less efficiently by bats (this is not known), but are probably more accessible to lemurs.

The phenology of section *Brevitubae* seems suited to mammal pollination. The dry season in Madagascar is a period of low availability of food (Petter

et al., 1975; Hladik et al., 1980), making baobab nectar a significant resource, and thereby encouraging visits by animals active at that time of year.

The rapid anthesis of bat-pollinated baobabs around dusk can be hypothesized to serve the biological role of reducing pollen and nectar loss to diurnal animals (e.g., bees and sunbirds) while allowing nectar presentation to primarily crepuscular paleotropical fruit bats (Baker, 1973; Marshall, 1983). However, the synchronization of anthesis with dusk seems less critical for lemur pollination, as both *Phaner furcifer* and *Cheirogaleus medius* foraged throughout the night.

SECTION *LONGITUBAE*

The pollination observations in section *Longitubae* suggest that these species manifest a radically different pollination system from that of the other sections. All species for which adequate data exist had long-tongued hawkmoths as their major pollinators. Bats were never involved and nocturnal lemurs were only observed to visit *A. rubrostipa* and *A. za*, where their visits did not contribute significantly to pollination. Nectarivorous birds were nectar thieves, although honeyeaters could be minor pollinators of *A. gibbosa* in Australia. Insects such as settling moths, ants, and bees were commensals or parasites, contributing no pollination. The pollination biology of *A. madagascariensis* remains unresolved, but long-tongued hawkmoths are the most likely pollinators given the general similarity of the floral morphology to *A. za* and other Malagasy *Longitubae*.

Section *Longitubae* has numerous characters that appear well suited to pollination by sphingids. The flowers are nocturnal and sweet-smelling and the androecium is pale and highly visible. The nectar is less copious than in the mammal-pollinated species and is well protected in the tubular calyx. The petal bases overlap, limiting access to the nectar, and the inner surface of the calyx has stiff, upward-pointing hairs making it difficult to obtain nectar from outside the petals. Instead, hawkmoths must obtain nectar by inserting the proboscis inside the petals (i.e., along the outside of the staminal tube) and into the nectar chamber through one of the five narrow openings between the petal bases. The concealment of the nectar means that short-tongued, settling insects can extract little, if any, nectar.

The flowers of section *Longitubae* are elongated, with a stigma-to-nectar distance similar to or longer than the proboscis length of the pollinating sphingids. As originally argued by Darwin (1862) and refined by Nilsson (1988), hawkmoths do not approach flowers closer than is necessary to acquire nectar. Hence, flowers significantly shorter than the moth's proboscis have no opportunity to deposit pollen on the insects' wings and bodies. Darwin (1862) also proposed that a hawkmoth whose proboscis is longer than the flower will be able to remove more nectar (but note Wasserthal's (1993) alternative explanation for the evolution of long proboscides). Taken together these forces can theoretically lead to a co-evolutionary "arms race" between sphingids and sphingophilous flowers which, it is argued, accounts for the extremes of proboscis and flower length found in some areas, including Madagascar. It seems likely that, as major nectar resources, Malagasy baobabs have played some part in this co-evolutionary spiral.

Relative to sections *Brevitubae* and *Adansonia*, section *Longitubae* has a low pollen : ovule ratio and highly versatile anthers. These features can each be hypothesized to be adaptive given the small size of hawkmoths relative to bats and lemurs. With a smaller surface area hawkmoths will become saturated with less pollen (see Heithaus et al., 1974) and with a smaller mass they will dislodge pollen from anthers less easily. Versatile anthers generally release pollen more easily than adnate or basifixed ones (note the tendency for versatile insertion in wind-pollinated plants) and thus are possibly adaptations to hawkmoth pollination.

Differences in androecial structure within *Longitubae* are hard to explain. However, the central bundle of *A. rubrostipa* can be viewed as a specialization that increases the number of anthers at the center of the flower where most hawkmoths hover while extracting nectar.

Wet-season flowering is usual in hawkmoth-pollinated plants, as the early wet season represents a peak in hawkmoth abundance and few moths are active during the dry season (Owen, 1969; Nilsson et al., 1985; Haber & Frankie, 1989).

As with bats, hawkmoths show a peak of activity soon after dark, probably due to temperature constraints on foraging (Cruden et al., 1976). Thus, like the mammal-pollinated species, the hawkmoth-pollinated baobabs benefit by rapid anthesis at dusk. The spectacularly rapid anthesis found in *A. rubrostipa*, *A. za*, and *A. madagascariensis* could have arisen through direct selection or alternatively as a developmental by-product of their extremely elongated flowers.

The Malagasy *Longitubae* differ strikingly from the remaining baobabs by their red and yellow floral

pigments. All four species have yellow or red petals, a red or pink inner calyx (whitish in some *A. perrieri*), and a red style. In searching for an adaptive hypothesis for this pigmentation it is noteworthy that many tropical hawkmoth-pollinated plants have reddish styles or filaments (Haber & Frankie, 1989). The limited studies of hawkmoth vision suggest that they see poorly at the red end of the visual spectrum (Knoll, 1922). It can thus be suggested that the red coloration "camouflages" the reproductive structures and thereby increases the frequency with which moths bump into them during nectar-feeding. Haber & Frankie (1989) made a similar suggestion to account for the presence of magenta filaments in some hawkmoth-pollinated *Calliandra* and *Capparis*. The red inner surface of the calyx can similarly be viewed as a means of discouraging insects from visiting the base of the flower and extracting nectar from outside the corolla (thereby avoiding pollination). The red petals of *A. madagascariensis* and the reddish blush on those of some *A. za* are, however, hard to explain adaptively, although the corolla plays a subsidiary role in the androecium in the visual display of section *Longitubae*. Likewise, it would be rash to hypothesize any adaptive value to the yellow corolla of many Malagasy *Longitubae*, as this could be a pleiotropic effect of the genes leading to a red style and calyx.

FUTURE WORK

Understanding the mechanisms that have led to floral diversity in *Adansonia* requires much further work. The pollination ecology of *A. madagascariensis*, *A. suarezensis*, and *A. grandidieri* would benefit from further study; additional studies of floral biology in these and other species would, likewise, be useful, especially on nectar production, nectar contents, and breeding systems. Also, studies of floral development would help evaluate phylogenetic/developmental constraint. Ultimately, such detailed understanding of the floral and pollination biology of extant baobabs and their close relatives in the Bombacaceae can be integrated with a robust phylogeny to gain insight into the relative roles of adaptation (direct selection), exaptation (co-option of characters for new roles), and developmental/phylogenetic constraint in the evolution of flowers.

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IN MEMORIAM

Peter Martin

20 June 1923–15 December 1994

Peter Martin shown here in New Zealand checking to see that *Alseuosmia* lives up to the translation of its name. Photograph by Beryl Martin.

Peter Gordon Martin was one of the most influential pioneers in the development of evolutionary studies using macromolecules, yet never really considered himself to be a plant systematist. He died peacefully on December 15, 1994, after a long struggle with cancer. He is survived by his wife of 48 years, Beryl (herself an internationally recognized artist), a son (Nick) and daughter (Mandy) and four grandchildren. His life and career were characterized by an enthusiasm and breadth of interests rarely seen in many researchers, and he imparted this to his colleagues and students.

Peter was born in Adelaide, South Australia, in 1923. He attended Unley Primary School and Prince Alfred College, joining the Royal Australian Navy in 1940, aged 17, as a midshipman, rising to the rank of lieutenant. Peter saw action in the Battle of the Coral Sea on HMAS Australia, and following training as a navigator and gunner at Portsmouth, U.K., served with HMS Keren at the allied landing in Sicily. In 1943, he joined HMAS Napier and was present in Tokyo Bay at the time of the Japanese surrender. He continued in service on HMAS Barwon and HMAS Gladstone until 1948. Between 1949 and 1951 he studied botany and genetics at the University of Adelaide, achieving First Class Honours in genetics in 1952, and his Ph.D. in 1955. He was awarded the Gowrie Trust Scholarship (1950); Elsie Marion Cornish Prize and Ernest Ayres Scholarship (both in botany, 1951); William Culrose Prize (1955) and Nuffield Dominion Scholarship (1958).

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In 1956 Peter was appointed as Lecturer in Biology at the University of Adelaide, and became Senior Lecturer in Zoology in 1959; Reader in Botany (1967); Professor of Botany (1969); and Professor Emeritus (1985) when he took early "retirement," aged 61, to devote his time fully to research on angiosperm molecular phylogeny—an area in which he continued to work as actively as possible right up to his death. He was also an active member of the South Australian Fauna and Flora Handbooks Committee.

As a university teacher Peter was remembered fondly by all of his students as someone whose enthusiasm for biology, and for evolution in particular, was as infectious as it was inspirational. He considered that to attract the best students to go on in higher years, it was essential that their introduction to biology in First Year be given only by the best lecturers in the university (although with characteristic modesty, he would deny his own exemplary lecturing ability). He was responsible for the introduction of genetics to the first-year biology curriculum at the University of Adelaide. Throughout his career he supervised numerous Honours students, as well as an M.Sc. and four Ph.D.s, in addition to inspiring and assisting numerous other students within the department, even well into his so-called retirement.

Peter's research spanned some 40 years and included work on microbial genetics, the role of the nucleus in the control of cell differentiation, marsupial chromosome structure and evolution, and angiosperm phylogeny and biogeography using plant protein, RNA, and DNA sequences. He was an early champion of evolutionary genetics, molecular phylogeny, and continental drift. At the Berlin International Botanical Congress in 1987, in addition to presenting the main paper in the session on "Macromolecular data in plant systematics: Proteins," he also stood up during question time at the session on cladistics and evolution, and announced that unless we all got involved in molecular sequencing we would soon find ourselves out of our jobs. How accurate his prophecy, given the current revolution in this field and the replacement in universities all over the world of traditional taxonomists by molecular biologists.

Peter's research experience included work at the John Innes Institute, Hertfordshire (1959–1960); Radio Biological Research Unit, Harwell, U.K. (1966); Department of Genetics, University of California–Davis; Royal Botanic Gardens, Kew, U.K. (1972, 1983); the Department of Botany, Durham University, U.K. (1976); and the Missouri Botanical Garden (1987). He maintained strong international research links with numerous colleagues and attended numerous conferences on plant systematics and molecular phylogeny. In addition to the Berlin Congress, he was an invited speaker at the Nobel Symposium on the "Hierarchy of Life" in Stockholm (1989); the 4th International Symposium on Plant Biosystematics (Kyoto, 1990); and the International Conference on the Systematics of the Euphorbiaceae (St. Louis, 1989). His career included some 48 papers in genetics, marsupial and angiosperm evolution, and biogeography. His long-running fascination was the relationships and biogeography of Gondwanan plant and animal groups. Among the many taxa which he studied were the Marsupialia, and the Proteaceae, Solanaceae, Podocarpaceae, and *Nothofagus*. He also investigated the molecular evolution of plant families that possess hemoglobin and/or fix nitrogen.

An avid reader and prolific writer, his passions were the history of Australian exploration, as well as the history and development of scientific thought. Peter was also a keen gardener and natural historian, influencing and often growing the subjects of Beryl's art. He was a natural teacher, providing his family and grandchildren with an endless source of information and inspiration. Peter will be remembered not only by his long-time friends and family, but also by the thousands of students for whom he made Biology come to life as a subject. His wide range of research interests and enthusiasm for research, and his unfailing encouragement for students, will be greatly missed. His opinions and solutions to problems (especially administrative ones), although not always shared by his colleagues, were nevertheless held genuinely and expressed forthrightly. Peter's openness, imagination, and originality placed him at the forefront of his field(s) and made him am inspiration to those who knew him.

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NOTICES

Jesse M. Greenman Award Now $1,000

The Greenman Award, a certificate and a cash prize of $1,000, is presented each year by the Missouri Botanical Garden. It recognizes the paper judged best in vascular plant or bryophyte systematics based on a doctoral dissertation published during the previous year. Papers published during 1994 are now being accepted for the 27th annual award, which will be presented in the summer of 1995. Reprints of such papers should be sent to Dr. P. Mick Richardson, Greenman Award Committee, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A. In order to be considered for the 1995 award, reprints must be received by 1 June 1995.

The Rupert Barneby Award

The New York Botanical Garden is pleased to announce that Ing. Nelson Zamora, of the Instituto Nacional de Biodiversidad (INBio), Costa Rica, is the recipient of the 1994 Rupert Barneby Award. Ing. Zamora will be working on several groups of mimosoid and caesalpinioid legumes for Costa Rica.

The New York Botanical Garden also invites applications for the 1995 Rupert Barneby Award. The award of $1,000.00 is to assist researchers planning to come to The New York Botanical Garden to study the rich collection of Leguminosae. Anyone interested in applying for the award should submit their curriculum vitae, a letter describing the project for which the award is sought, and how the collection at NYBG will benefit their research. Travel to NYBG should be planned between January 1, 1996 and January 31, 1997. The letter should be addressed to Dr. Enrique Forero, Director, Institute of Systematic Botany, The New York Botanical Garden, Bronx, NY 10458-5126 USA, and received no later than December 1, 1995. Announcement of the recipient will be made by December 15th. Anyone interested in making a contribution to THE RUPERT BARNEBY FUND IN LEGUME SYSTEMATICS, which supports this award, may send their check, payable to The New York Botanical Garden, to Dr. Forero.

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Two Important New Titles from the Missouri Botanical Garden

Moss Flora of Central America
Part 1. Sphagnaceae–Calymperaceae

Bruce Allen with contributions from H. Crum, R. A. Pursell, W. D. Reese & N. Salazar Allen

Central America possesses one of the world's richest moss floras, with an estimated 871 species in an area only one-half that of Colombia, which has about as many species. Previous to this publication the only moss flora for any Central American country was E. B. Bartram's now nearly half-century-old *Mosses of Guatemala*.

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Marshall R. Crosby & Robert E. Magill

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Annals of the Missouri Botanical Garden

FIRST INTERNATIONAL CONFERENCE ON THE RUBIACEAE: INTRODUCTION[1]

Charlotte M. Taylor[2]

The first International Conference on the Systematics of the Rubiaceae was organized to bring together workers in this family from around the world for the first time, and give an overview of ongoing research in all of its aspects. The event was held at the Missouri Botanical Garden in St. Louis on 4–6 October 1993, and was organized by C. M. Taylor and P. Mick Richardson.

The Rubiaceae are estimated to include about 10,700 species in 637 genera (Robbrecht, 1988), making them the fourth most speciose family of flowering plants. Members of this family are found worldwide (except Antarctica), from cold to warm and wet to dry climates; the cosmopolitan genus *Galium* L. is found in most of these habitats. Members of the Rubiaceae are predominantly woody, although numerous species are herbaceous. The majority of genera are tropical, with most of their species concentrated in wet forests. Within these forests, the Rubiaceae are usually an important component of the woody vegetation (Gentry, 1988). The pantropical genus *Psychotria* L. is the most speciose in the family, with at least 700 species, and is also one of the largest genera of flowering plants. Cronquist (1981) classified the Rubiaceae in the Asteridae, in the order Rubiales, along with the Theligonaceae, which included only the genus *Theligonum*. He considered the Rubiales to be related to the Gentianales (in particular Loganiaceae) and Dipsacales (in particular Caprifoliaceae). More recent analyses based on *rbc*L sequences (Chase et al., 1993) place the Rubiaceae among the families of the Gentianales, but not near the Dipsacales. Robbrecht (1988) treated *Theligonum* as a member of the Rubiaceae, in the tribe Theligoneae of the subfamily Rubioideae.

Contemporary work on the systematics of the Rubiaceae is based largely on the thorough summary of morphology and previous systematic work and the proposed classification by Robbrecht (1988). More detailed evaluation of the tribes and subfamilies proposed there, as well as of tribes and genera of uncertain placement, have already resulted in some modifications to this system (see, for example, the papers by Bremer et al. and Andersson in this issue), and more work is under way.

Presentations made at the conference addressed many aspects of the Rubiaceae, at various levels. Surveys of morphological features across the entire family were presented by C. Puff ("Overview of

[1] I am grateful to Carol Davit for her invaluable help in organizing this conference, and to the Missouri Botanical Garden for institutional support.
[2] Missouri Botanical Garden, P. O. Box 299, St. Louis, Missouri 63166, U.S.A.

the Rubiaceae"), M. Kiehn ("Chromosome survey of the Rubiaceae"), and D. Bridson ("An annotated list of morphological terms/descriptors used in the Rubiaceae—A first stage towards the standardization of terminology"). Another morphological presentation considered the anatomy and biology of bacterial leaf nodules found in some members of this family (I. M. Miller, "Bacterial leaf nodulation in the Rubiaceae: An enigmatic relationship"). Several surveys of morphological features, notably palynological ones, were presented for particular geographic regions (S. L. Jung-Mendaçolli, "Pollen morphological studies in Brazilian Rubiaceae"; C. T. Chen, "Pollen morphology of Chinese Rubiaceae") or tribes (S. M. Pire, "Palynology of the Tribe Spermacoceae"; C. Persson, "Pollen and exotesta morphology in Gardenieae–Gardeniinae"). Classification and phylogenetic reconstruction were considered for the family as a whole (B. Bremer, "Phylogeny of the Rubiaceae mainly based on chloroplast DNA data (*rbc*L sequences and restriction site data)"; E. Robbrecht, "Formal approaches to the classification of Rubiaceae"), for entire tribes (L. Andersson, "Tribes and genera of the Cinchoneae complex"; J.-F. Manen & A. Natali, "Phylogeny of the Tribe Rubieae: Evidence from chloroplast DNA"), for tribes as represented in particular geographic regions (N. M. Bacigalupo & E. Cabral, "Delimitation of the American genera of the Tribe Spermacoceae"; C. F. Puttock, "The Gardenieae of Australia and the western Pacific"), and for individual genera (D. H. Lorence, "Systematics and evolution of *Randia* (Gardenieae) in Mexico"; C. Gustafsson, "*Randia* (Gardenieae) in South America"; I. A. Schanzer, "Taxonomy of southwestern Asian *Galium* L. sect. *Orientigalium* Ehrend."; A. Borhidi, "Problems and views in the taxonomy of the *Rondeletia* complex"; S.-M. Chaw, "The delimitation and geographical origin of paleotropical *Antirhea*"; S. P. Darwin, "Recent progress in the taxonomy of *Timonius* in Papuasia").

The five articles that follow were developed from presentations made at the conference and are arranged here in the order in which they were presented there: Puff et al. survey unusual arrangements in the androecia of various genera, all involving fusions or agglutinations, which they describe in morphological and anatomical detail. These modifications vary in structure and have apparently arisen independently. They have been little remarked and never described completely before. Kiehn presents for the first time a summary of the karyological data for this family, most of it original, arranged by subfamily and tribe. He discusses the level of karyological variation for each of these taxa, and the contributions that these data make to our understanding of relationships within the family. Bremer et al. test previously proposed classifications of the Rubiaceae at the subfamilial and tribal level and also look at the relationships of several problematic genera, based on DNA sequence data for selected genera. This is one of the first analyses of *rbc*L sequences to address the classification of the Rubiaceae as a whole, and the results variously support or contradict details of previous classifications. Andersson tests the circumscription of the Cinchoneae and closely related tribes, a problematic complex, using morphological characters. He presents a revised circumscription of three tribes, comments on individual genera, and proposes two new genera. Natali et al. test the circumscription and relationships of the Rubieae using chloroplast DNA sequence data. They describe two unique deletions that characterize monophyletic groups in the subfamily Rubioideae, and their analysis shows several distinct clades within the Rubieae and suggests that several genera as currently circumscribed may be polyphyletic.

The Second International Rubiaceae Conference, convened by E. Robbrecht, E. Smets, and C. Puff, is scheduled for 12–14 September 1995 at the National Botanic Garden of Belgium.

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THE UNITED STAMENS OF RUBIACEAE. MORPHOLOGY, ANATOMY; THEIR ROLE IN POLLINATION ECOLOGY[1]

Christian Puff, Anton Igersheim, Ralf Buchner, and Ulrike Rohrhofer[2]

Abstract

Based on morphological and anatomical investigations of selected species of *Argostemma, Steenisia, Strumpfia, Acranthera, Antherostele,* and *Posoqueria,* and reports in the literature about several other taxa, the paper surveys how unions in the androecium of Rubiaceae can come about. In *Argostemma,* the formation of anther tubes is either by union of adjacent apical connective appendages and agglutination of neighboring pollen sac walls, or only by union of connective appendages (with or without sterile remnants of pollen sacs); the anthers dehisce longitudinally and the subepidermal cell layer (endothecium) has thickenings. *Argostemma,* moreover, has species without proper anther tubes in which the anthers (with apical pores and a pollen sac wall without thickenings in the subepidermal cell layer) are, nevertheless, variously grouped together. The anther tube of *Steenisia* comes about by the interlocking of epidermal layers of neighboring apical connective appendages and agglutinated cuticles. *Strumpfia* is unique in having a special cell layer uniting all anthers. The strictly cylindrical anther tube of *Antherostele* is, like in the Asteraceae, held together by agglutinated cuticles of neighboring pollen sacs; apical connective appendages are not involved in the tube formation. *Acranthera* has an "anther-style and stigma complex" (stamens, united by apical connective appendages, are firmly connected with the stigma; descriptions and comments about the highly unusual stigmatic areas in this genus are also included). *Posoqueria* has an anther head, which is held together only by agglutinated, sterile appendages or extensions of the thecae that are produced both basally and apically. The unstable nature of the anther complex is a prerequisite for proper function of *Posoqueria's* unique "catapult mechanism" of the stamens, by which pollen is hurled at the pollinator. *Argostemma, Steenisia, Neurocalyx,* and *Strumpfia* are believed to be buzz pollinated. In taxa with longitudinal anther dehiscence (*Argostemma* in part, *Steenisia, Neurocalyx*), the apically constricted anther tubes with porelike openings are thought to represent structures analogous to the poricidally dehiscent anthers of buzz-pollination systems. It is speculated that at least some *Acranthera* species may also be buzz pollinated; the windows formed by the elongated connective appendages above the longitudinally dehiscent anthers might take over the function of pores.

Agglutinations or fusion in the androecium, or unions by other means, are not confined to the "Synandrae" (= Asteridae sensu stricto) but also occur in various other families, including a number of Rubiaceae. In one rubiaceous genus, there is even an "anther-style and stigma complex." These phenomena are often imprecisely and vaguely described as "anther tube," "anther cone," "anthers convergent," "anthers connivent," or "anthers connate."

One of the aims of the present paper is to show the range of variation of the "synandry" syndrome in the Rubiaceae, which apparently has evolved independently in various alliances. Detailed morphological and anatomical documentation, based on the investigation of preserved material and supplemented by field observations, will be presented for selected taxa.

The presence of united anthers ("united" is used here in a neutral and wide sense, i.e., presence of agglutinations, proper fusion, or union by other means in the androecium), as well as shape, size, color, presentation, and orientation of the entire androecium, appears to be intimately linked with pollination biological specializations.

Materials and Methods

Detailed morphological and anatomical studies were carried out on the following taxa and collections (material preserved in 70% alcohol or FPA; vouchers deposited in WU, unless stated otherwise). Methods used for SEM (scanning electron microscope) investigations and microtome techniques are described in Puff et al. (1993) and Igersheim (1993a). **: carried out on reconsti-

[1] This research was supported by the "Fonds zur Förderung der Wissenschaftlichen Forschung" (Project P8260-BIO). Thanks are extended to W. Dvorak for help with the plates.
[2] All: Institute of Botany, University of Vienna, Rennweg 14, A-1030 Wien, Austria.

tuted herbarium material (for details on method see Buchner & Puff, 1993).

Acranthera Arn. ex Meisn. (Isertieae?, incertae sedis): *A. atropella* Stapf: Borneo. Sabah: Tawau Hills Park, *Puff & Buchner 920419–1/4* (Fig. 11; 11A from color slide Puff SEA-1887). *A. frutescens* Val.: Borneo. Brunei: Temburong Distr., *Puff & Buchner 920506–1/1* (Fig. 10; 10A from color slide Puff SEA-1643). *A. involucrata* Val.: Borneo. Brunei: Temburong Distr., *Puff & Buchner 920506–1/2* (Figs. 8, 9A–E; 8A from color slide Puff SEA-1649). *Acranthera* sp., aff. *A. variegata* Merr. (subg. *Dichroanthes* Bremek.): Borneo. Brunei: Temburong Distr., *Puff 900818–1/9* (Fig. 9F, G).

Antherostele Bremek. (Urophylleae): *A. banahaensis* (Elmer) Bremek.: Philippines. Luzon: Sorsogon Prov., Mt. Bulusan, *Elmer* 16166 (US)**. *A. grandistipula* (Merrill) Bremek.: Philippines. Luzon: Camarines, *Sulit B.Sc. 30316* (A)** (Fig. 12).

Argostemma Wall. (Argostemmateae): *A. borragineum* Bl. ex DC.: Borneo. Sabah: Crocker Range, *Puff 890705–1/3* (Figs. 1, 2). *A. diversifolium* Ridl.: Thailand. Peninsula, Trang Prov., forests behind Kachong Bot. Garden, *Puff 900910–1/6* (Fig. 5); Surat Thani Prov., just outside Khao Sok National Park, *Puff & Sridith 930705–1/1* (Fig. 4C, from color slide Puff TH-1915). *A. moultonii* Ridl.: Borneo. Sabah: Crocker Range, Mt. Alab, *Puff 890705–1/2* (Fig. 4A, from color slide Puff SEA-125). *A. neurocalyx* Miq.: Thailand. Peninsula, Krabi Prov., Khao Phanom Distr., Khao Phanom Bencha National Park, *Puff & Sridith 930731–1/2* (Fig. 6A–C, G, H); Surat Thani Prov., Khao Sok National Park, *Puff & Sridith, 930703–1/7* (Fig. 6D–F, I). *A. pictum* Wall.: Thailand. Peninsula, Surat Thani Prov., just outside Khao Sok National Park, *Puff & Sridith 930705–1/2* (Fig. 4D, from color slide Puff TH-1872). *A. psychotrioides* Ridl.: Borneo. Brunei: Belait Distr., *Puff 900807–1/1* (Fig. 3B), 900810–1/9 (Fig. 3A, C–F). *Argostemma* sp.: Thailand. Peninsula, Phattalung Prov., Ban Phut Distr., Khao Bantad Wildlife Sanctuary, Pri Wan waterfall, *Puff & Sridith 930725–1/1* (Fig. 4B, from color slide Puff TH-2220).

Posoqueria Aubl. (Gardenieae subtribe Gardeniinae): *P. latifolia* (Rudge) R. & S.: Origin unknown, cult. Indonesia, Bot. Garden Bogor, *Puff 920913–1/s.n.* (Fig. 13, from color slides Puff SEA-2214, SEA-2216); cult. National Bot. Garden Belgium sub 64–0515, as *P. panamensis* (Walp. & Duchass.) Walp. (Fig. 14).

Steenisia Bakh. f. (Rondeletieae): *S. pleurocarpa* (Airy Shaw) Bakh. f.: Borneo. Brunei: Temburong Distr., *Puff 900818–1/12* (Fig. 7; 7A from color slide Puff SEA-1276).

Strumpfia Jacq. (incertae sedis): *S. maritima* Jacq.: Cuba. Santiago de Cuba, between Daiquiri and Siboney, *Igersheim 920603–1/1* (Fig. 4E, from color slide Igersheim CU-92/78).

Non-rubiaceous taxa: *Ardisia* sp. (Myrsinaceae): Borneo. Brunei: Temburong Distr., *Puff 920506–1/s.n.* (Fig. 4F, from color slide Puff SEA-1657). *Solanum* sp. (Solanaceae): Colombia. Los Nevados Nat. Park, *Puff 920220–1/s.n.* (Fig. 4G, from color slide Puff CL-201).

Results

ARGOSTEMMA (FIGS. 1–3, 4A–D, 5, 6)

Argostemma is a relatively large genus (ca. 150 spp.) with diverse floral characteristics. Corolla shapes vary from the "common" subrotate corollas to bell-shaped types. The anthers are either dehiscent longitudinally or open by means of apical pores. In the former group, the anthers typically have (long) apical connective appendages, and the anthers form a solid tube. In taxa with poricidal dehiscence the anthers may or may not be connate, and apical connective appendages are absent.

Comprehensive investigations have so far not been available for any *Argostemma* species, and the present investigation must restrict itself to a few selected examples. Thus data here presented may represent only a small, possibly incomplete selection of the diverse situation in the genus.

Species with longitudinal dehiscence of the pollen sacs and anthers united into a tube, as exemplified by A. borragineum *and* A. psychotrioides.

Argostemma borragineum has a straight anther tube, and its general flower shape is similar to that of *A. moultonii* (Fig. 4A). The anther tube is distinctly 5-ridged due to the presence of prominent, rounded connectives, especially from the upper

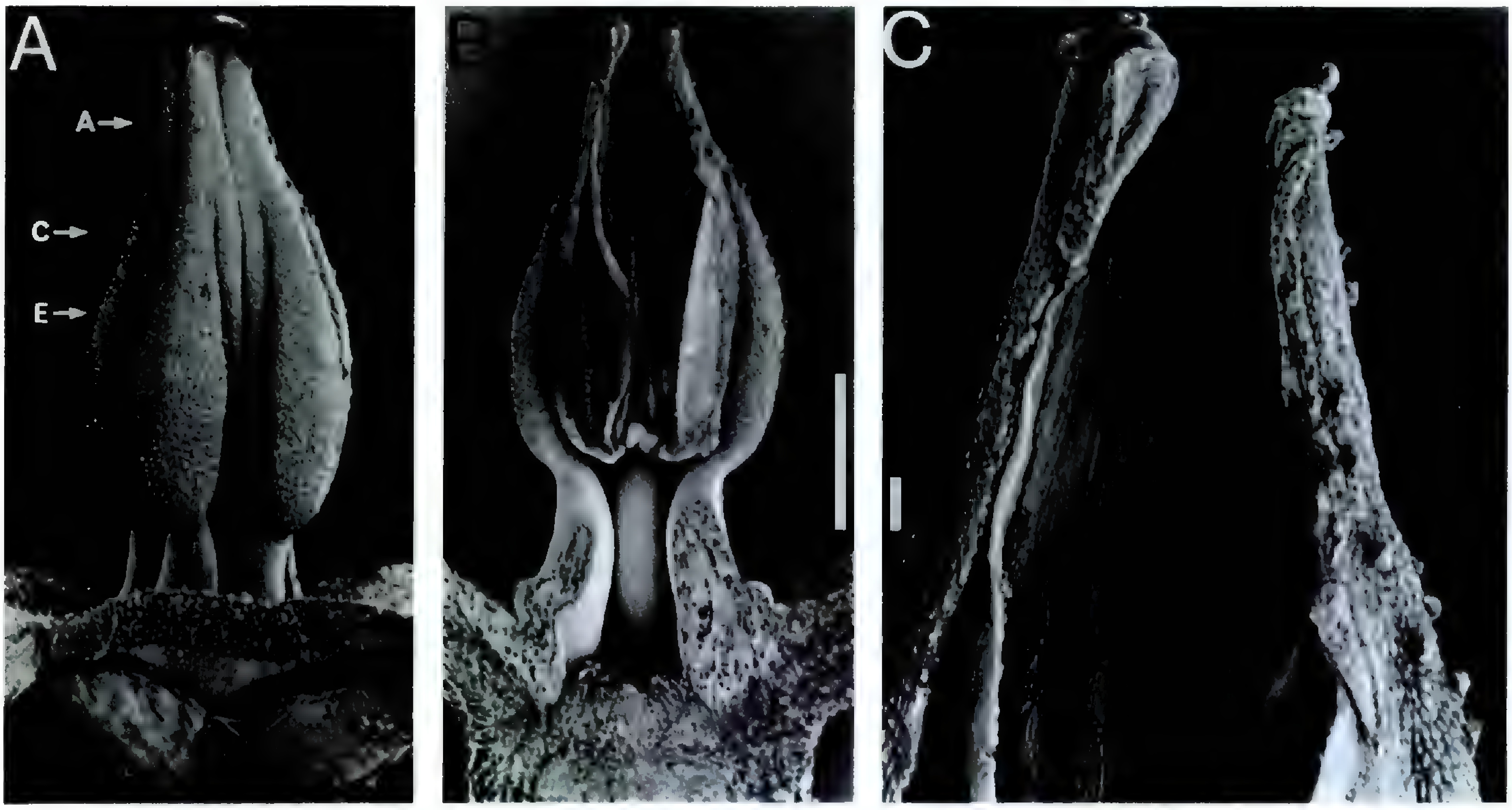

Figure 1. *Argostemma borragineum.*—A. Calyx and corolla removed to show anther tube; the arrows indicate the approximate levels of the sections reproduced in Figure 2.—B. Section of anther tube to show longitudinal dehiscence of pollen sacs and apical appendages.—C. Detail of upper part of anther tube; note free ends of apical connective appendages and upward extensions of pollen sacs. SEM-graphs. Scale bars: 1 mm (A = B); 0.1 mm (C).

third of the anther tube downward (Figs. 1A, 2E, F). The sequence of sections reproduced in Figure 2C–F shows the shape change of the connectives and the pollen sacs. The filaments are very short and somewhat broadened near the base.

The apical part of the anther tube is comprised of sterile anther appendages. The majority of the volume of these apical appendages is made up of prolonged connective tissue, with a distinct "inner" and "outer" epidermis and some layers of parenchymatic tissue between (Fig. 2B). On either side of each angled connective appendage are sterile pollen sac extensions (Fig. 2A, also cf. Fig. 1B, C). These sterile upward extensions of the pollen sacs appear to reach the apex of the anther tube, but they should not be mistaken for dehiscent pores. On the contrary, the pollen sacs open by means of longitudinal slits, and pollen is released into the interior of the anther tube (Figs. 1B, 2D–F).

The entire anther tube forms a solid, united structure. It is always the epidermal layer of pollen sacs from neighboring anthers, which, over a restricted, narrow area, are connected with each other by their fused cuticles (Fig. 2), even in the uppermost, sterile part of the tubes. Detailed study of the pollen sac wall reveals the presence of an endothecium with ringlike thickenings (Fig. 2G).

The fine structure of the anther tube of *Argostemma psychotrioides* differs from that of *A. borragineum* in certain details:

The apical parts of the anther tube are comprised of connective appendages only; there are no sterile extensions of pollen sacs (Fig. 3A). The apical appendages are united in a different way than in *A. borragineum*: The epidermis of the connective appendages develops elongated outgrowths, which are uniseriate but often two- or even three-celled. These outgrowths of neighboring connectives are interlocked, and in between them there is always an intensively stained, mucilaginous substance (Fig. 3B).

The section shown in Figure 3C goes through the uppermost parts of the pollen sacs, which already contain pollen grains. Still, the connection of neighboring anthers by means of interlocked elongated, fingerlike epidermis outgrowths is the same as higher up on the anther tube.

Nearer to the base of the anther tube (Fig. 3D), these specialized cells are no longer present. The epidermal cells of pollen sacs of neighboring anthers are merely in close proximity to each other, but an obvious unification is not detectable (Fig. 3E). As in *A. borragineum*, the endothecial layer shows ringlike thickenings. Tangential sections (Fig. 3F) show that the shapes of the endothecium cells vary from more or less rectangular to fusiform within the same pollen sac wall. It is hardly possible to correlate cell shape and the kind of thickening with the four types of endothecial cells Bremer (1989) distinguished in Bornean *Argostemma* species.

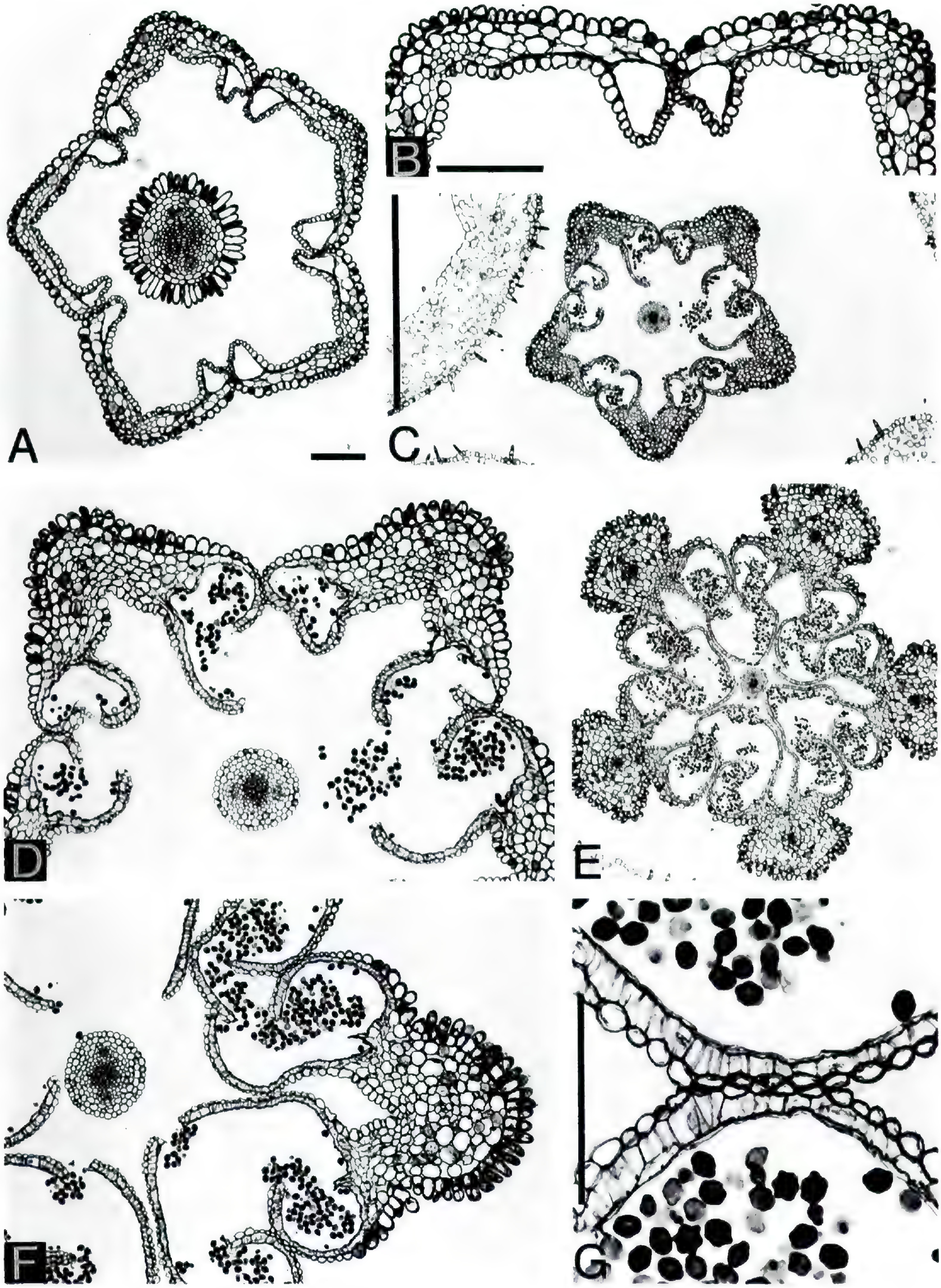

Figure 2. *Argostemma borragineum*, cross sections of the anther tube.—A. Apical connective appendages with a sterile pollen sac remnant on either side, the stigma in the center (elongated peripheral cells: stigmatic papillae).—B. Detail of A, showing agglutination of adjacent connectives and sterile, terminal portion of pollen sacs.—C. Above the middle of the anther tube; as compared to lower down (cf. E), pollen sacs still small in size.—D. Detail of C, pollen sacs of adjacent anthers agglutinated; note shape of connectives.—E. Approximate middle of anther tube; note prominent, dorsally rounded connectives.—F. Similar to E; note dehisced pollen sacs.—G. Agglutinated pollen sacs

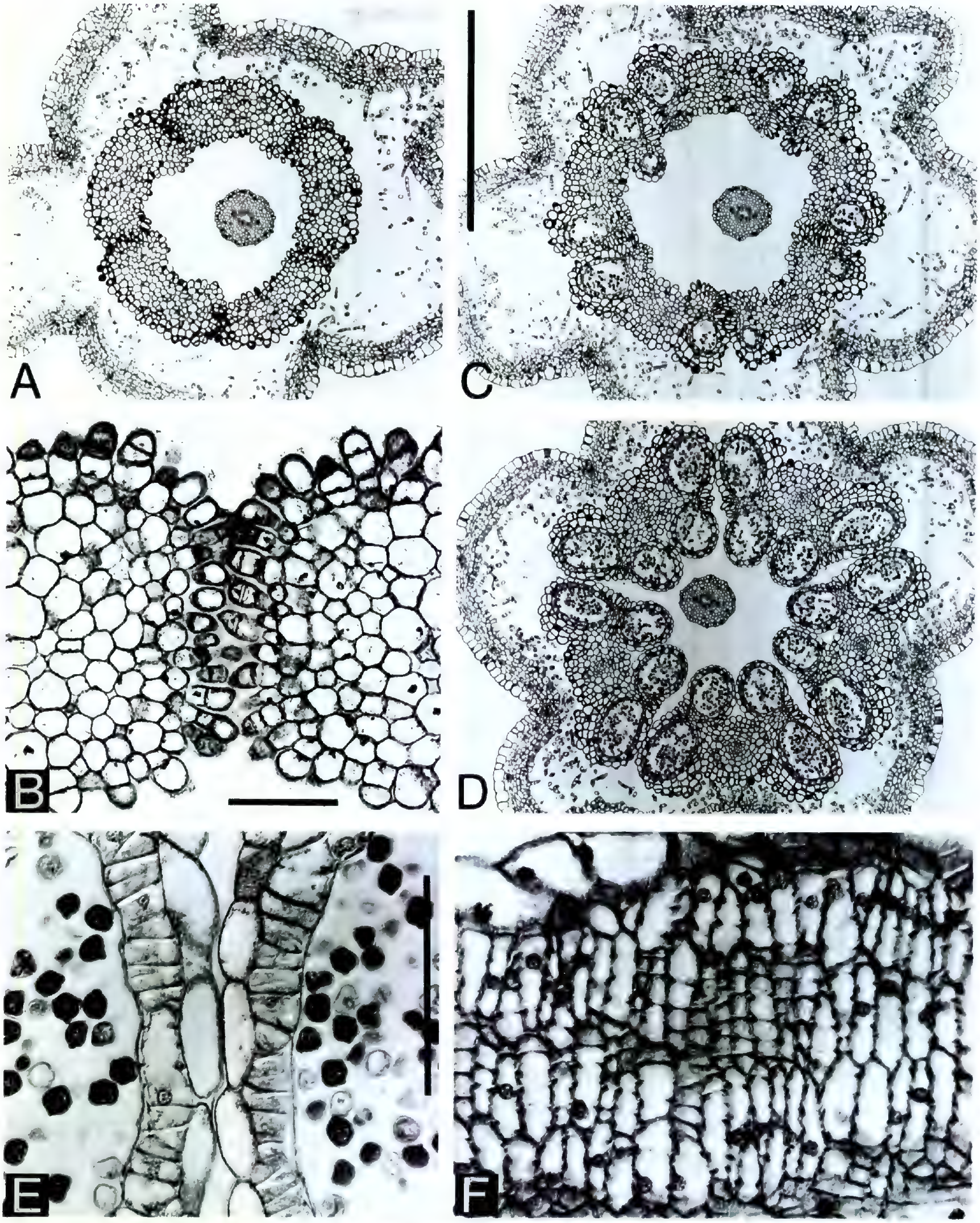

Figure 3. *Argostemma psychotrioides.* A–E, Cross sections of flower buds shortly before anthesis. —A. Uppermost part of anther tube; ring formed by apical connective appendages (corolla outside; style in the center). —B. Interlocked cells of two adjacent connectives. —C. Lower level than A, the uppermost parts of the pollen sacs are visible on either side of the connective. —D. Approximate middle of anther tube; note unequal size of adaxial and abaxial pollen sacs. —E. Detail of pollen sacs of two adjacent anthers, no obvious agglutination is discernible; note thickenings in the endothecium. —F. Tangential section of endothecium. A–F: LM-graphs, E–F: Differential Interference Contrast. Scale bars: 1 mm (A = C = D); 0.1 mm (B; E = F).

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of adjacent anthers; note thickenings in the endothecium. A–G: LM-graphs (light micrographs). Scale bars: 1 mm (C = E); 0.1 mm (A = D = F; B: G).

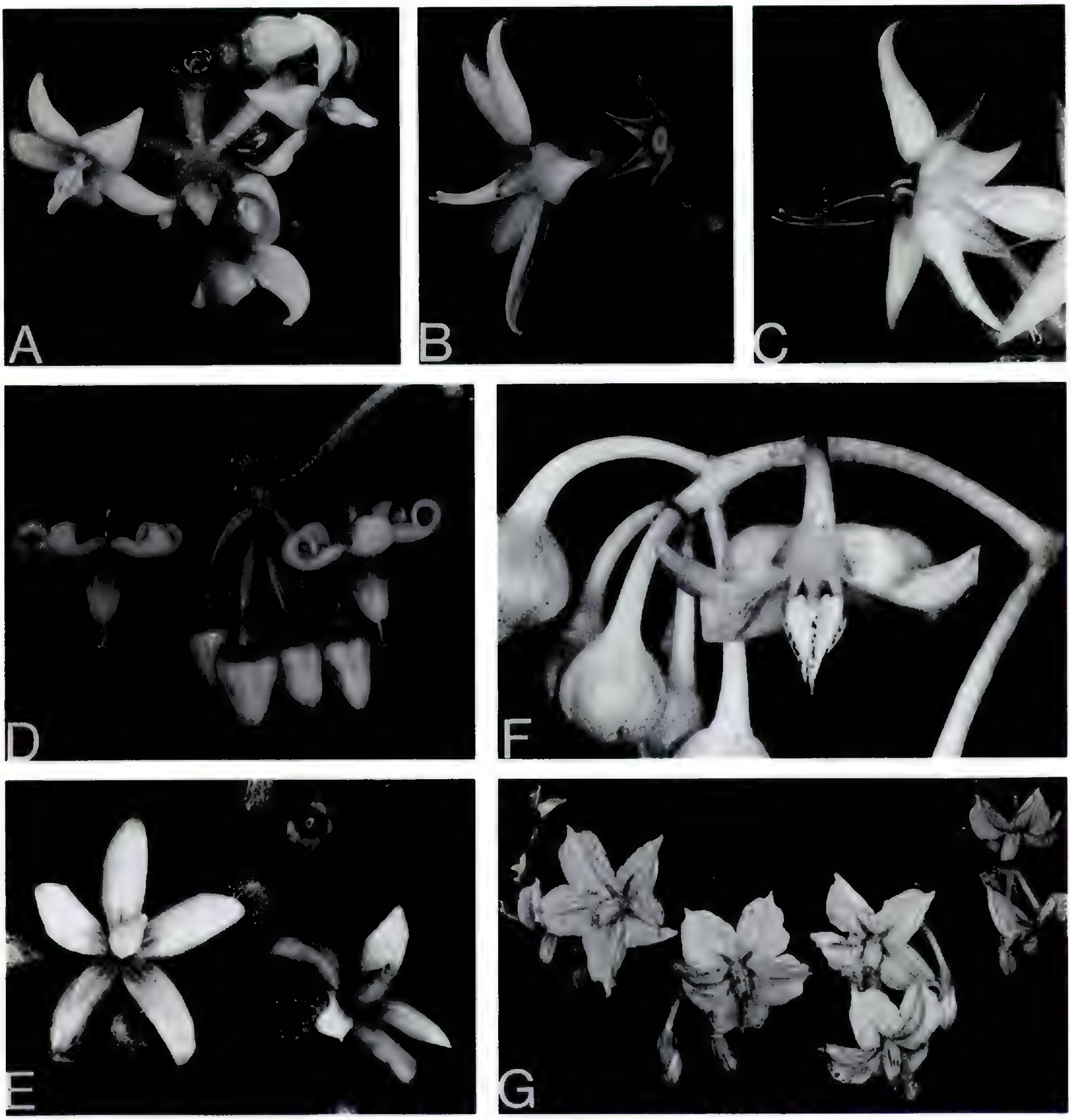

Figure 4. A–D, Flowers of *Argostemma.*—A. *A. moultonii.*—B. *Argostemma* sp.—C. *A. diversifolium.*—D. *A. pictum.*—E. Flowers of *Strumpfia maritima.* F, G. Buzz-pollinated flowers of non-rubiaceous taxa.—F. *Ardisia* sp. (Myrsinaceae).—G. *Solanum* sp. (Solanaceae).

Also note the shape of the anthers in cross section (Fig. 3D): as compared to *A. borragineum* (Fig. 2E), the rounded connectives are rather small, and the abaxial pollen sacs of each anther are roughly in the same plane as the connective. Consequently, the anther tube is not a 5-*ridged* structure (ridges = connectives) as in *A. borragineum,* but more or less regularly 5-angled. Such a 5-angled anther tube is also found in *A. pictum* (Fig. 4D).

Species with poricidal dehiscence of the pollen sacs and (a) connivent anthers and (b) a more or less zygomorphic anther arrangement, as exemplified by A. diversifolium *and* A. neurocalyx, *respectively.*

The anthers of *Argostemma diversifolium* are held in a tubelike arrangement (Fig. 4C), but no fusions or agglutinations are present. The arrangement of the androecium often is slightly zygomorphic because all five filaments have an S-like curvature (thus moving the base of the anthers away from the center of the flower), and because the anthers themselves are also a little curved (Fig. 4C). Such slight curvatures also occur in *Argostemma* species with solid anther tubes, long, sterile

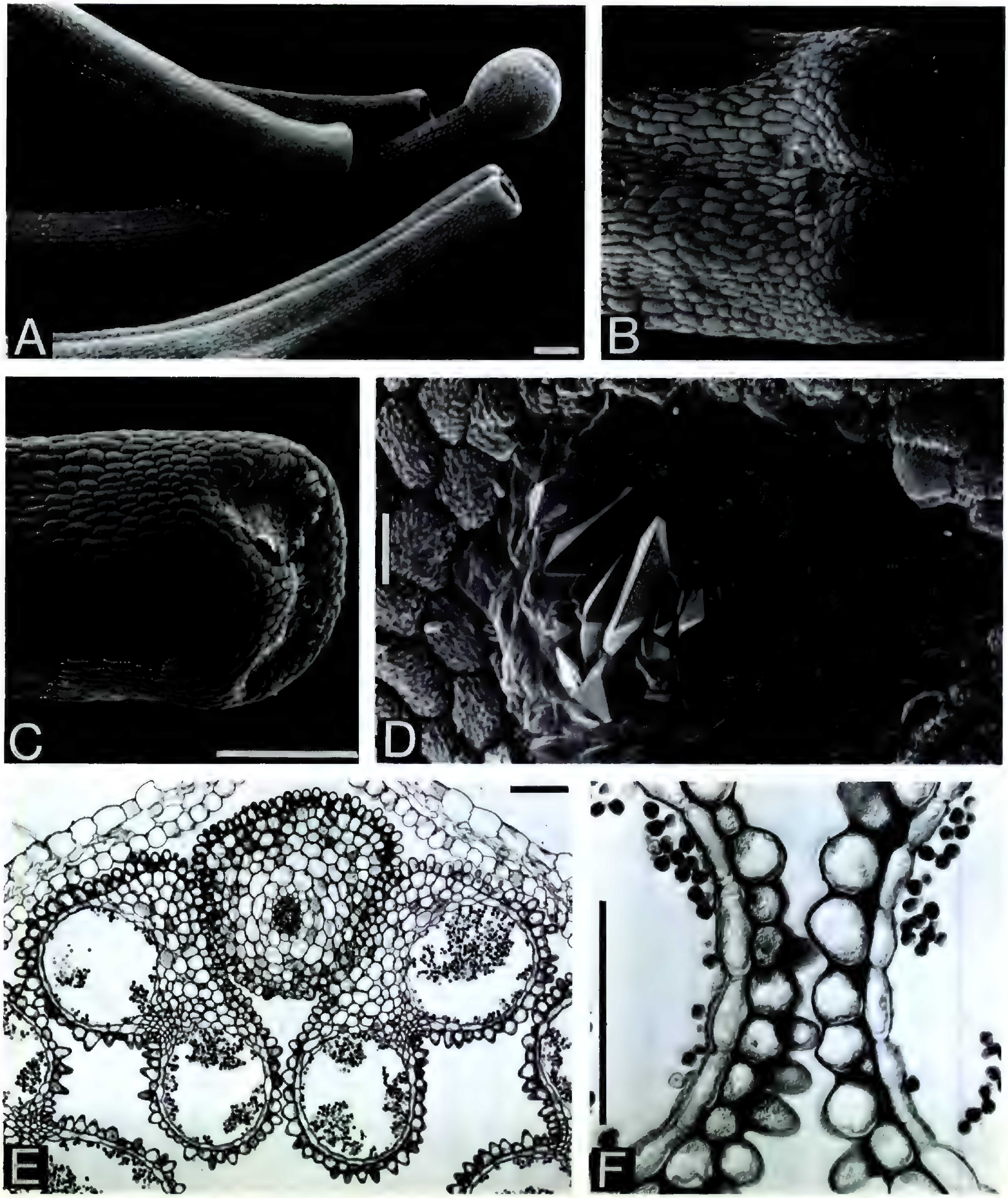

Figure 5. *Argostemma diversifolium.*—A. Upper parts of stamens, style, and stigma (compare with Fig. 4C).—B. Poricidal dehiscence of anther (from open flower).—C. Apical part of anther (from bud) showing areas where the pores will be formed.—D. Detail of pore-forming area; note calcium oxalate crystals.—E. Cross section of anther near its base (bud shortly before anthesis, note corolla outside the anther).—F. Detail of pollen sacs of two adjacent anthers; note absence of thickenings in the endothecium. A–D: SEM-graphs; all others: LM-graphs. Scale bars: 0.1 mm (A; B = C; E; F); 10 μm (D).

apical appendages, and longitudinal dehiscence of the pollen sacs; the unidentified species from peninsular Thailand depicted in Figure 4B is a good example.

In this species, the anthers are prolonged for some distance below the insertion point of the filaments (so the anthers are not strictly basifixed as, for example, in *A. borragineum*, cf. Fig. 1B, or

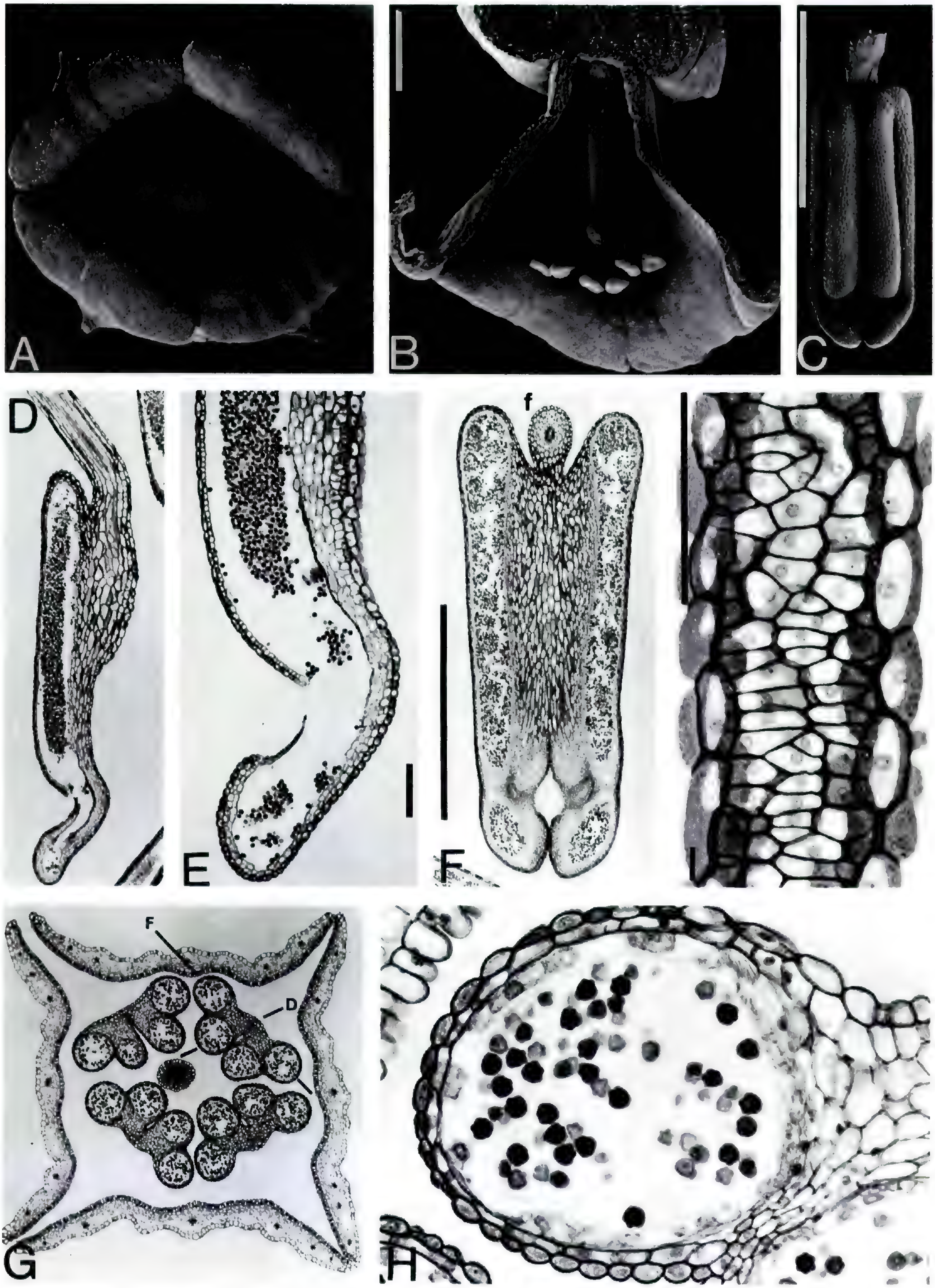

Figure 6. *Argostemma neurocalyx.*—A. Front view of flower; note slight zygomorphy, enhanced by the position of the anthers (the fourth, slightly shorter stamen is not clearly visible).—B. Longitudinal section of flower, one stamen removed.—C. Isolated solitary stamen; note spoonlike structure formed apically by the two abaxial pollen sacs.—D. Off-median longitudinal section of stamen (approximate level of section indicated in G).—E. Detail of apical part of anther; note dehiscence pore.—F. Longitudinal section of stamen showing section of up-turned filament (f), connective, and abaxial pollen sacs (approximate level of section indicated in G).—G. Cross section of bud shortly before anthesis

A. moultonii, Fig. 4A) and are sagittate. Cross sections of the basal portions of the anthers thus show the free filament and a theca (= paired pollen sac) on either side of it (Fig. 5E). Above the insertion point of the filament, the connective is inconspicuous and occupies a relatively small area relative to the thecae. Apically, each anther has two round pores (Fig. 5A, B). In young anthers, the areas where the pores will be formed are easily recognizable as thin-walled slight depressions with large calcium oxalate crystals on their surfaces (Fig. 5C, D).

In contrast to the *Argostemma* taxa with longitudinally dehiscing pollen sacs, the pollen sac walls of *A. diversifolium* lack an endothecium with thickenings; there is merely a parenchymatic layer of "normal" cells below the pollen sac epidermis (Fig. 5F).

Argostemma neurocalyx represents yet another type of *Argostemma* flower. The 4-merous flower itself has a slightly zygomorphic, bell-shaped corolla (Fig. 6A). The arrangement of the anthers, all held near the two basal corolla lobes and in close proximity to each other, further enhances the zygomorphy (Fig. 6A, B). In nature, the flowers are held in a horizontal position.

There is no actual cellular fusion in the androecium. The abaxial and adaxial pollen sacs of each theca are of unequal lengths. The former are notably longer than the adaxial ones and form a kind of spoonlike structure (Fig. 6C) because their apical parts are slightly upturned (Fig. 6D, E). The abaxial side of the anthers is flat because abaxial pollen sacs and connectives are in one plane (cf. Fig. 6G). Whereas the abaxial pollen sacs are separated by the connective tissue (Fig. 6F, G), the adaxial ones are closer to each other (i.e., in a more central position), immediately above the connectives. These anthers each have two pores (i.e., each theca has one pore), which is common to one adaxial and one abaxial pollen sac. Because the abaxial pollen sacs are longer, this common pore is located some distance away from the anther apex, i.e., in the lowermost part of this spoonlike structure. Cross and tangential sections of the pollen sac wall (Fig. 6H, I) show the presence of a subepidermal, parenchymatic cell layer, but an endothecium, in the sense of a cell layer with fibrous thickenings, is absent.

Comments on anther dehiscence and endothecium.

The present investigations on *Argostemma* species suggest that there is a clear correlation between longitudinally dehiscent anthers and the presence of an endothecial layer with fibrous thickenings on the one hand, and the absence of thickenings in the subepidermal cell layer of the pollen sac wall in taxa with poricidal anther dehiscence on the other hand.

The suppression of the endothecium appears to be the rule in plants whose anthers open by pores (Goebel, 1923), and this phenomenon occurs both in monocots and dicots, notwithstanding their familial position and relationships (Ericaceae, Solanaceae, etc.). *Argostemma*, however, needs to be added to the relatively few examples where both situations occur in the same genus.

Comments on the pollination ecology.

Argostemma flowers belong to the "*Solanum*-type" of pollen flowers sensu Vogel (1978) (Fig. 4G). This "*Solanum*-type" is widespread and occurs in oligandrous families and orders, and in both monocots and dicots (including *Ardisia* spp., Myrsinaceae; Fig. 4F). Characteristics of the "*Solanum*-type" are, according to Vogel (1978), that the few, mostly (sub)sessile anthers are enlarged, thus becoming more showy and capable of producing excess pollen. The powdery pollen is released in small portions as a "cloud" by vibrating movements of the visitors, thus dusting their bodies. These "*Solanum*-type" flowers are strictly melittophilous and often (but not always) zygomorphic (compare the actino- and zygomorphic *Argostemma* species depicted in Fig. 4A–D), and the visitor clasps the androecium in a fixed position. Because of the overall dusting of the visitor, the position of the small stigma is independent from that of the anthers. In view of the widespread occurrence of floral nectaries in the Rubiaceae (normally produced on the "roof" of the inferior ovary), the absence of nectaries in *Argostemma* is noteworthy and congruent with the "*Solanum*-type" of pollen flowers.

As noted above, vibratile ("buzz") pollination is characteristic, and Buchmann (1978) does in fact include *Argostemma* in his table of buzz-pollinated

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(note corolla lobes); sections of the upper half of the anthers.—H. Detail of pollen sac; the cell layer below the pollen sac epidermis has no thickenings.—I. Tangential section of endothecium; note absence of the thickenings. A–C: SEM-graphs; all others: LM-graphs. Scale bars: 1 mm (A = B; C; D = F = G); 0.1 mm (E; H = I).

angiosperms. Buchmann (1978, 1983), however, overemphasized the intimate link between buzz pollination and poricidally dehiscent anthers, and Vogel (1978) also mentioned only poricidal anthers as being a characteristic of "*Solanum*-type" flowers.

We believe that those *Argostemma* species with longitudinally dehiscent anthers and an anther tube are also buzz pollinated. The formation of an apically constricted tube leads to the same effect as apical pores because there is always a terminal opening; the exserted style never entirely blocks this tube but always leaves enough room for pollen to exit. Support for this interpretation comes from Sazima et al. (1993: 80), who noted, "It must also be kept in mind that flowers with longitudinal slits may form, using other morphological elements, tubular structures analogous to the buzz anthers." In his study of *Lycopersicon* (Solanaceae), a genus which, like many *Argostemma* species, has solid anther tubes with sterile apical appendages and longitudinally dehiscent anthers, Teppner (1993) provided actual photographic proof of buzz pollination by bees.

A (far from complete) survey of *Argostemma* species shows that the presumably buzz-pollinated species show great diversity in shape, size, color, and arrangement of the androecium and also in the shape and outline of the (most probably always) white corolla.

In some species (e.g., *Argostemma moultonii*, Fig. 4A), all floral parts are pure white, or in others (e.g., *A. chaii*, *A. neurocalyx*, Fig. 6A) white except for the yellowish or yellow to pale orange stamens (both filaments and anthers). In *A. pictum* (Fig. 4D), the anthers are yellowish above, but basally the connectives and short filaments are green. Not uncommon are green spots near the base of the corolla lobes in addition to the green filaments, yellow anthers, and whitish apical connective appendages (e.g., *A. yappii*; Bremer, 1984: fig. 1C: color illustration). *Argostemma diversifolium* (Fig. 4C) may be the only species with blue anthers, green filaments, and green spots near the insertion points of the filaments. In the *Argostemma* species depicted in Figure 4B, the swollen, somewhat enlarged filaments are orange (dark in the b/w reproduction), and anthers as well as apical connective appendages are very pale yellow to whitish.

In all but one of the examples given above, the corolla shapes range from subrotate to subcampanulate, and the lanceolate corolla lobes are spreading to recurved or reflexed. *Argostemma neurocalyx* has a small (ca. 5 mm long) campanulate, slightly compressed (and, therefore, slightly zygomorphic) 4-merous corolla with short recurved lobes, and the entire appearance of the flower is reminiscent of that of certain Gesneriaceae, such as small-flowered *Didymocarpus* species, also because all four anthers are located in the vicinity of the "lower lip."

STEENISIA (FIG. 7)

Existing information on the androecium of this small genus (5 species, all occurring in Borneo) is superficial. Bremer (1984) described its condition only as "anthers adnate to an ovate cone; thecae with two loculi; connective produced apically above the thecae" (p. 337) and "thecae open by vertical slits" (p. 334); the relevant illustrations are too imprecise to draw any definite conclusions.

The species studied, *S. pleurocarpa*, has a subsessile, pale yellow anther tube (the free filaments < 0.5 mm long) which is ca. 4–5 mm long. Only the lower two-thirds or less of the entire tube is occupied by the pollen sacs, whereas the "apical" remainder is exclusively formed by flattened connective appendages (Fig. 7B, D). Each of these appendages is, on average, 5–6 cells thick and contains a weakly developed vascular bundle in the center (Fig. 7E). The most apical parts of these appendages are rounded and free from each other. Slightly below the apex, however, the connective appendages form a solid, not easily ruptured, angled tube or hollow cone. The neighboring appendages are interlocked in a peculiar fashion (Fig. 7F); they are fused by their cuticles. Similarly, the epidermis cells of pollen sacs of neighboring anthers are united by fusion of their cuticles in the lower half of the anther tube (Fig. 7G, H). Consequently, the entire anther tube, from bottom to top, is a solid unit.

Upon maturity, the pollen is released into the interior of this tube. The pollen sacs dehisce longitudinally. The line, where the pollen sacs will dehisce, is already clearly visible in late bud stage (Fig. 7H, triangle). The endothecium of the anthers has ringlike thickenings (visible in some cells, but not yet fully developed in the anther section depicted in Fig. 7H; from a bud shortly before anthesis; note that tapetum remnants are still present).

According to Bremer's (1984) descriptions and illustrations, *S. pleurocarpa* has 5-merous flowers and white corollas. However, the study material and other populations of this species seen in Borneo all had only 4-merous flowers (cf. Fig. 7G) and pink corollas.

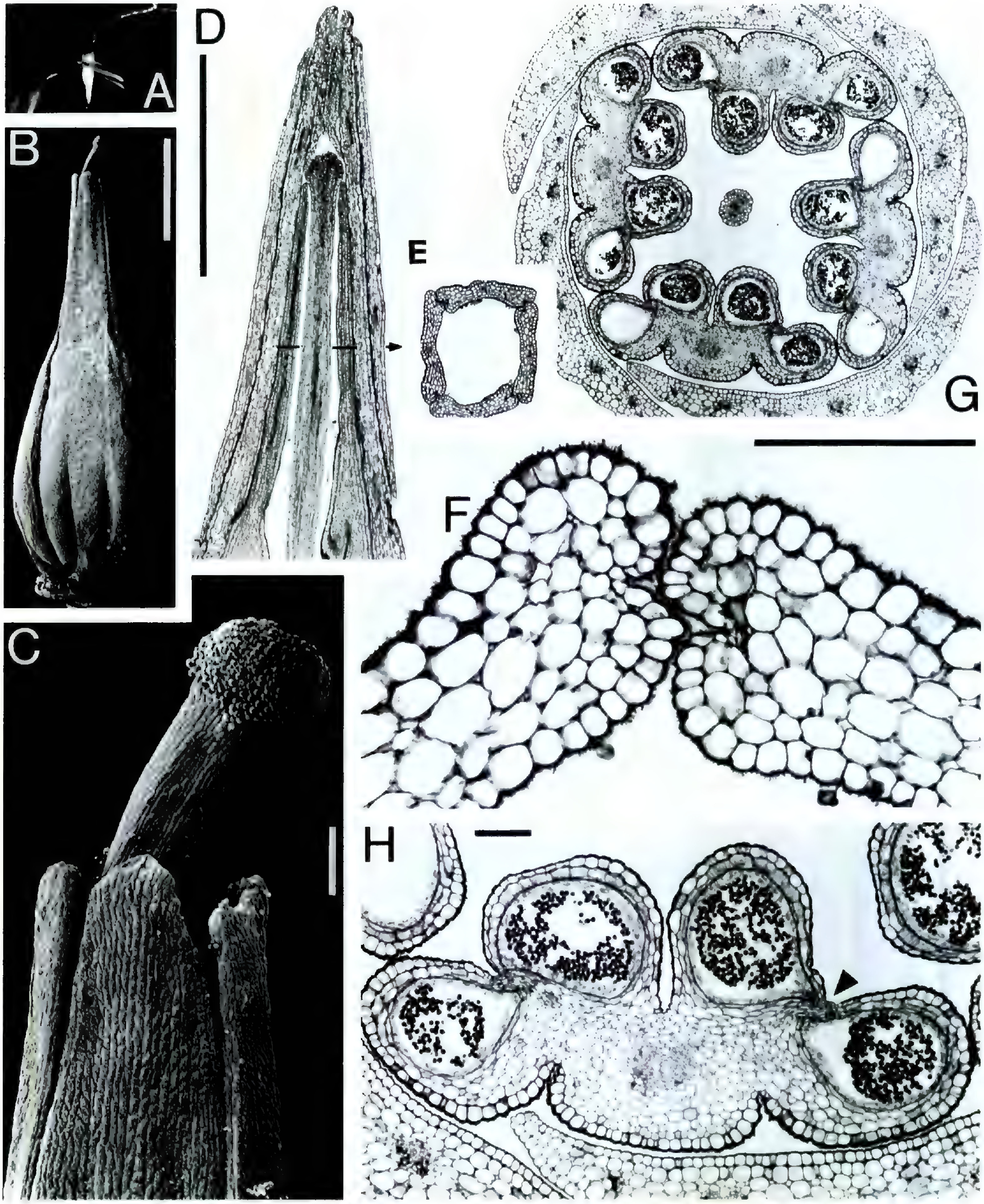

Figure 7. *Steenisia pleurocarpa.*—A. Open flower in natural, pendulous position (corolla pink; anther cone pale yellow, style and stigmas white).—B. Anther cone of flower at anthesis, corolla removed.—C. Detail of A, showing free ends of apical connective appendages, and the exserted, always slightly curved style and stigma.—D. Longitudinal section of uppermost part of bud shortly before anthesis; visible from out- to inside: corolla, connective appendages and uppermost part of anthers (below), style, and stigma.—E. Cross section of connective appendages at the level indicated in D.—F. Detail of E, interlocked connective appendages of two neighboring stamens.—G. Cross section through the lower third of a flower bud shortly before anthesis (from out- to inside: corolla lobes, anthers, style).—H. Detail of G; the lateral pollen sacs of the anther in the center are in close contact but not fused with those of the neighboring anthers (triangle: area of pollen sac dehiscence). A and B: SEM-graphs; all others: LM-graphs. Scale bars: 1 mm (B; D = E = G); 0.1 mm (C; F; H).

Comments on the pollination ecology.

There can be no doubt that the flowers of all *Steenisia* species, like those of *Argostemma*, belong to the buzz-pollinated "*Solanum*-type" of pollen flowers sensu Vogel (1978). Variation in corolla shape and size and androecium color and arrangement is not as great as in *Argostemma*, and the corollas are generally star-shaped, ranging only in color from white to pink. The entire androecium (anther tube plus filaments) is pale yellow to orange (cf. Bremer, 1984: fig. 1A, color illustration of *S. borneensis*). As in *Argostemma*, a nectar-producing disk is absent.

In all but one *Steenisia* species the styles are beset with upwardly directed hairs (cf. relevant figures in Bremer, 1984). These hairs do not extend to the uppermost part of the style but are generally confined to the region that is at the same level as the pollen sacs. It seems plausible to conclude that their function is to "catch" released pollen.

It should also be noted that in all *Steenisia* species the upper part of the style invariably is curved (Fig. 7A, C; also cf. relevant illustrations in Bremer, 1984). The pollination biological relevance of this feature may not be great; compare in this context the information given on general characteristics of "*Solanum*-type" of pollen flowers under *Argostemma*.

STRUMPFIA (FIG. 4E)

This monotypic Caribbean genus stands out among all other Rubiaceae with fusions or agglutinations in the androecium in that it is the only known taxon in which all five anthers are united into a tube by means of a discrete cell layer (a kind of "super epidermis"). Refer to Igersheim (1993a) for illustrations and detailed information on additional peculiarities such as the formation of partially united, crownlike apical connective appendages, the retraction of the style in the functionally male stage, and the proterogyny in this genus.

Presumably on the basis of literature data, Buchmann (1978) had included the genus in his list of buzz-pollinated taxa. One of us (Igersheim, in prep.) is now able to provide proof and photographic documentation for the occurrence of buzz pollination.

ACRANTHERA (FIGS. 8–11)

Acranthera is an Asiatic genus of ca. 35 species, whose taxonomic position to this day is not entirely resolved. The reason for this unsatisfactory situation is the lack of detailed data on most of its character states. In floral characteristics, the genus is rather diverse, as reflected by Bremekamp's (1947) infrageneric subdivision into seven subgenera, whereby some of these are further split into "series."

The present investigation is restricted to three of Bremekamp's subgenera: (1) Subgenus *Phanerochiton* Bremek. (*A. involucrata*) and (2) subgenus *Dichroanthes* Bremek. (*Acranthera* sp., aff. *A. variegata*), both of which are distinguished by an elongated "receptaculum pollinis" sensu Bremekamp; the two are primarily separated by inflorescence characters. (3) Subgenus *Mitracme* Bremek., characterized by a mitriform "receptaculum pollinis" sensu Bremekamp that is much shorter than the thecae; the four "series" he recognized within the subgenus are distinguished by "eciliolate" or "ciliolate connectives" (e.g., series Ciliolatae: *A. frutescens*), and by inflorescence shape (umbel-like inflorescences: series Umbelliflorae: *A. atropella*).

The genus *Acranthera* is characterized by the presence of united apical connective appendages. Invariably, only the very apical parts of these appendages are fused (e.g., Figs. 8H, 10E). Microtome sections (Fig. 9A, B) reveal that this unification comes about by epidermal cells of neighboring connective appendages being interlocked, coupled by the fusion of their cuticles.

The length of the sterile, apical connective appendages varies from short (e.g., *A. involucrata*, Fig. 8E, G) to relatively long (e.g., *A. frutescens*, Fig. 10C). Appendage length is obviously correlated with the size and shape of the stigmatic areas (see below for details).

The (typically) 5, elongated and rather narrow anthers have dorsally prominent connectives that are either rounded (cf. Figs. 8F, 10A, B, 11C) or pointed (triangular in cross section, Fig. 9F, G). Viewed from above, the anther complex is a 5-lobed structure (Figs. 8A, 10A, 11C).

In the investigated species, the anthers either have hairs only in the border area between connective and pollen sacs (i.e., a line of hairs laterally on either side of the connective; *A. frutescens*, Fig. 10B, D), or also on the pollen sacs (*A. involucrata*, Fig. 8B, F, G). Bremekamp's (1940) distinguishing character, "ciliolate connectives," therefore, is rather imprecise.

Not only in the studied, but presumably in all taxa of the genus, the pollen sacs open with longitudinal slits (Figs. 8G, 10C, D). Pollen is thus released into the interior of the anther complex.

All taxa covered by the present study have very

Figure 8. *Acranthera involucrata* (subg. *Phanerochiton*).—A. Pinkish, short-tubed flowers with exposed, white anther complexes.—B. Anther-style and stigma complex from young bud; note apical connective appendages. C–E. From bud older than in B.—C. Anther-style and stigma complex, several anthers removed to show style and stigma.—D. Isolated style and stigma (anthers removed). Receptive areas not yet fully developed; compare with H.—E. Two isolated stamens held together by apical connective appendages; style and stigma removed. Note hairs on the pollen sacs. F–H. From open flower.—F. As B, but note "hairy windows" between anthers.—G. As E, but pollen sacs dehisced.—H. Detail of the apical region; note fusion between apical connective appendages and the stigma (triangle), and the receptive surface of the stigma. A–H: SEM-graphs. Scale bars: 1 mm (B; F = G; C = D = E); 0.1 mm (H).

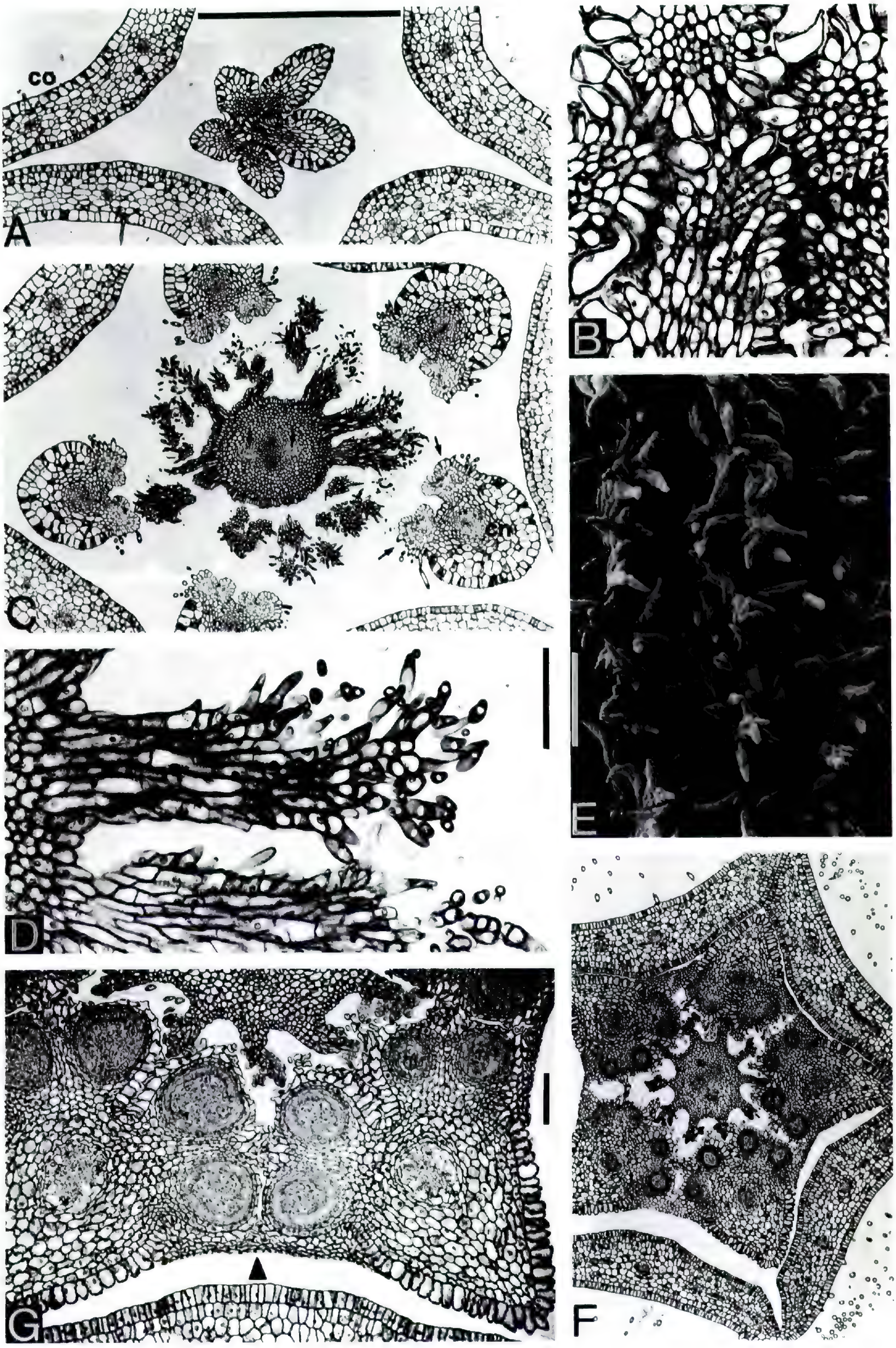
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Figure 10. *Acranthera frutescens* (subg. *Mitracme*, series *Ciliolatae*).—A. Greenish yellow, ± bell-shaped flower from above; the whitish anther complex is included in the tubular part of the corolla.—B. Anther-style and stigma complex from open flower; note "windows" above pollen sacs.—C. Isolated anther; note dehisced pollen sacs and long apical connective appendage.—D. Upper part of anther-style and stigma complex, one stamen removed to show mitriform stigma.—E. Columnar tissue (triangle) connecting stigma with connective appendages.—F. Detail of stigmatic area (receptive surfaces). A–F: SEM-graphs. Scale bars: 1 mm (B = C; D); 0.1 mm (E; F).

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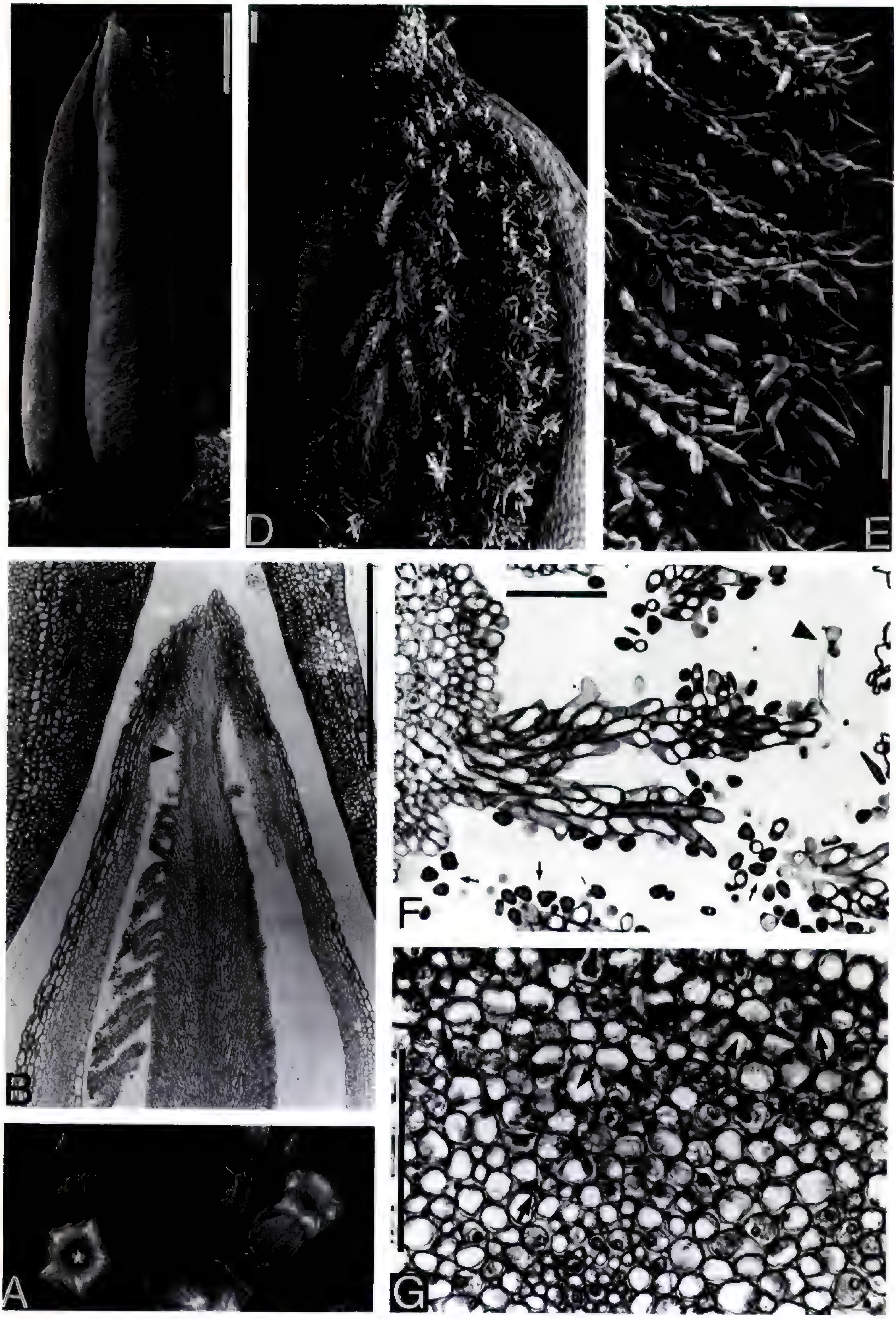
Figure 9. A–E. *Acranthera involucrata* (subg. *Phanerochiton*). A–D. Cross sections of bud shortly before anthesis.—A. Uppermost part, united connective appendages (co, corolla lobe).—B. Detail of A, showing tissue fusion of connective appendages.—C. Slightly lower level than A, showing connectives (cn) with sterile, uppermost parts of the thecae (arrows) and fingerlike, branched stigmatic areas (arrows in the central column of the stigma point to loose cells, which most probably correspond to the region where the receptive stigmatic papillae or hairs of a "typical" bicarpellate Rubiaceae with two stigma lobes would be located).—D. Detail of "stigma-fingers" with branched endings.—E. Rows of branched stigmatic papillae. F, G. *Acranthera* sp., aff. *A. variegata* (subg. *Dichroanthes*), cross sections of bud shortly before anthesis.—F. Corolla, anthers and stigmatic "fingers."—G. Detail of F, two neighboring anthers still connected by fragile, parenchymatic tissue (triangle; this will tear at anthesis, resulting in the formation of longitudinal slits between anthers). E: SEM-graph; all others: LM-graphs. Scale bars: 1 mm (A = C = F); 0.1 mm (B = D; E; G).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short (Figs. 8F, G, 10B) or subobsolete filaments (Fig. 11C). This is not uniformly so in the genus; see comments below.

A unique feature of *Acranthera* is that the apically united connective appendages are, in turn, united with the stigma by means of a columnar tissue (Figs. 8H, 10E, 11B, D). The latter is a solid, parenchymatic tissue, which is an upward extension of the central portion of the stigmatic tissue (Fig. 11B). This intimate connection between connective appendages and stigma was already present in the youngest floral developmental stages available for investigation (i.e., seemingly very young buds), indicating that this fusion, although undoubtedly postgenital in nature, must come about at very early stages of development.

The presence of this "anther-style and stigma complex" prompted Bremekamp (1947: 237) to state "[This] is a feature so entirely unparalleled in the Rubiaceae, that one may feel inclined to regard the position of the genus with regard to the rest of the family, as similar to that of the Asclepiadaceae with regard to the Apocynaceae, although in this way the importance of the connection between the anthers and the style is doubtless overemphasized."

The major misconception in Bremekamp's (1947) monograph of *Acranthera* is that he continually emphasized the presence of a "receptaculum pollinis." He stressed (p. 271) that "the pollen is temporarily deposited on the upper part of the style, which, to that end, is covered by rows of papillae."

The detailed studies discussed below prove that the situation is entirely different.

In *Acranthera involucrata*, style and stigma form a 10-ridged, uniform columnar structure (Fig. 8C, D; from a young bud). At this stage of anthesis no part of the style or stigma is visible from outside. The anthers bear relatively short apical connective appendages so that, in contrast to other investigated species, the anther complexes of open flowers do not show conspicuous apical "windows" (= gaps between connective appendages; Fig. 8F, compare with Figs. 10B and 11C).

The 10 ridges of the style and stigma column "fit" perfectly into the gaps between pollen sacs of neighboring anthers, and in the depressions between the two adaxial pollen sacs of each anther (cf. *Acranthera* sp., aff. *A. variegata*, Fig. 9F).

In bud stage, the upper half of the style and stigma column bears indistinct (not yet fully developed) hairlike appendages (Fig. 8C, D) on the ridges. During further development (from buds shortly before anthesis onward), the ridges grow out into fingerlike structures, and their irregularly arranged, hairlike appendages, whose ultimate parts are uniseriate and uni- or pluricellular, become very prominent (Fig. 9C, D). Figures 8H and 9E show such rows of irregularly arranged, hairlike appendages. Cross sections from the upper third of this style and stigma column reveal that the "central core" always shows two vascular bundles and, at a right angle to them, two areas (or sometimes only a single, uniform area) of looser cells (Fig. 9C; also cf. *Acranthera* sp., aff. *A. variegata*, Fig. 9F).

The interpretation of the hairlike appendages borne on the fingerlike structures described above is that they must represent the actual receptive (stigmatic) areas. Two stigma lobes as such (bicarpellate gynoecium) are never developed. The loose cells perpendicular to the two vascular bundles (see above) most probably correspond to the region where the receptive stigmatic papillae or hairs of a "typical" bicarpellate Rubiaceae with two stigma lobes would be located (cf., for example, *Crocyllis*, Puff & Mantell, 1982: fig. 15).

Acranthera is thus unique in the Rubiaceae in having an "anther-style and stigma complex" and also in the structure of its stigmas.

In the Rubiaceae a "receptaculum pollinis," or pollen-presenting organ, is typically the nonreceptive style, or style plus the nonreceptive outside of the actual stigmas, both of which may be provided with special adaptations (hairs, sticky substance, enlarged, specialized structures; cf. Igersheim, 1993b: fig. 1). Moreover, this mechanism of secondary pollen presentation is most probably always linked with protandry: the stigma lobes only unfold

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Figure 11. *Acranthera atropella* (subg. *Mitracme*, series *Umbelliflorae*).—A. Pale yellow, ± bell-shaped flowers; the whitish anther complexes are included in the tubular parts of the corollas.—B. Longitudinal section of uppermost part of bud shortly before anthesis; visible from out- to inside: corolla, connective appendages, stigmatic area, and columnar tissue connecting apical connective appendages and stigma (triangle). C–G. Open flower.—C. Corolla removed to show anther-style and stigma complex; note stigmatic areas protruding from windows above pollen sacs.—D. Stamen removed to show mitriform stigma.—E. Stigma "fingers" with receptive hairs.—F. Cross section of stigma "finger"; note pollen grains (small arrows) and germinating pollen grain (triangle).—G. Cross section of the central column of the stigma with numerous pollen tubes (arrows). B, F, and G: LM-graphs; C–E: SEM-graphs. Scale bars: 1 mm (B; C); 0.1 mm (D; E; F; G).

and become receptive after the pollen has been shed (and carried away by pollinators). In view of this, *Acranthera*'s "receptaculum pollinis" sensu Bremekamp (1947) is not comparable to that of other Rubiaceae.

While in the *Acranthera* species described above, the unusual stigmatic areas may, to some extent, also function to "catch" pollen from its own anthers, their main and primary function is to receive "foreign" pollen. At this stage, there is, unfortunately, no information on whether the species is self-compatible or self-incompatible.

In *Acranthera frutescens* and *Acranthera atropella*, it is more obvious that the stigmatic (receptive) areas have nothing to do with secondary pollen presentation, and definitely do not function as a "receptaculum pollinis." In both species, the apical connective appendages are relatively long, and the gaps between the individual appendages form five "windows" above the pollen sacs (Figs. 10B, 11C).

During later stages of anthesis (i.e., after the pollen sacs have dehisced), the stigmatic areas protrude from these windows (Fig. 11C). As compared to *A. involucrata* (and all species of subg. *Phanerochiton* and *Dichroanthes*), the stigmatic areas are short and mitriform, their lengths corresponding to the lengths of the apical "windows" (Figs. 10D, 11D). As in the previously described species, there is no indication of the presence of two "normal" stigma lobes.

The stigmatic "fingers," moreover, differ in size and fine structure. In both *A. frutescens* and *A. atropella*, they are considerably longer than in *A. involucrata*. *Acranthera frutescens* has shorter stigmatic "fingers" than *A. atropella*, and each "finger" bears numerous, short hairlike appendages that are arranged over the entire surface (Fig. 10F). In *A. atropella*, the elongated, obliquely downward-directed "fingers" (Fig. 11D, E) are sometimes forked near their base (Fig. 11F). Terminally, the "fingers" often bear pluricellular, uniseriate hairlike appendages, which frequently are in an irregularly starlike arrangement.

In *Acranthera frutescens* and *A. atropella*, stigmatic areas and the region of the pollen sacs are clearly spatially separated. It is therefore not possible that the stigmatic areas can serve as a "receptaculum pollinis" for pollen from its own anthers. When the pollen is shed, it must drop to the bottom, i.e., on to the roof of the inferior ovary. From the viewpoint of pollination ecology (see also below) it is relevant to stress that in all *Acranthera* species investigated by us a nectar-producing disk (in Rubiaceae normally developed on the roof of the ovary) was absent.

In *A. atropella*, we frequently detected in SEM-graphs and microtome sections that numerous pollen grains are caught in between the stigmatic areas. Sometimes, grains even showed pollen tubes (Fig. 11F). A detailed investigation of the central, columnar core of the stigmatic area often revealed the presence of numerous pollen tubes in the intercellular spaces of the parenchymatic tissue (Fig. 11G). We believe that these flowers had been visited by pollinators, and that the pollen and pollen tubes present are from another flower. We do not have proof of the presence of a self-incompatibility system, however.

Comments on the pollination ecology.

In spite of the fact that actual field observations are lacking, several comments on pollination ecological aspects seem appropriate.

It is believed that the investigated *Acranthera* species, especially those of subgenus *Mitracme* (*A. frutescens* and *A. atropella*), are buzz pollinated.

The size and shape of the nectarless flowers, as well as of the "anther-style and stigma complex" (< 10 mm long), agree well with "typical" oligandrous buzz-pollinated flowers. The five apical windows (*A. frutescens* and *A. atropella*) could be likened to apical pores of buzz-pollinated taxa with convergent anthers. It should be noted in this context that initially, i.e., immediately after the dehiscence of the pollen sacs, these windows are not yet obscured by the protruding stigmatic areas; the latter are still considerably smaller at this stage (flowers protandrous). It is, in our opinion, easy to imagine that a visiting insect can vibrate pollen out of the interior of the "anther-style and stigma complex."

Vibratile pollination may also work in taxa of subgenera *Phanerochiton* and *Dichroanthes*, both characterized by elongated stigmatic areas. It is possible that pollen is equally well "vibrated" out of the longitudinal slits between the anthers as out of the apical "windows" of the taxa of subgenus *Mitracme*.

As mentioned before, the floral features of *Acranthera* are diverse, and it is probable that the floral biology may be entirely different in some of the subgenera not investigated in detail.

Although characterized by anther-style and stigma complexes similar to those described above, the two "northwestern" subgenera, subgenus *Acranthera* (as "*Eu-Acranthera* " in Bremekamp, 1947; Sri Lanka and Indian Peninsula) and subgenus *Androtropis* (India (Assam) and peninsular Thailand), differ from other *Acranthera* taxa in some essential ways:

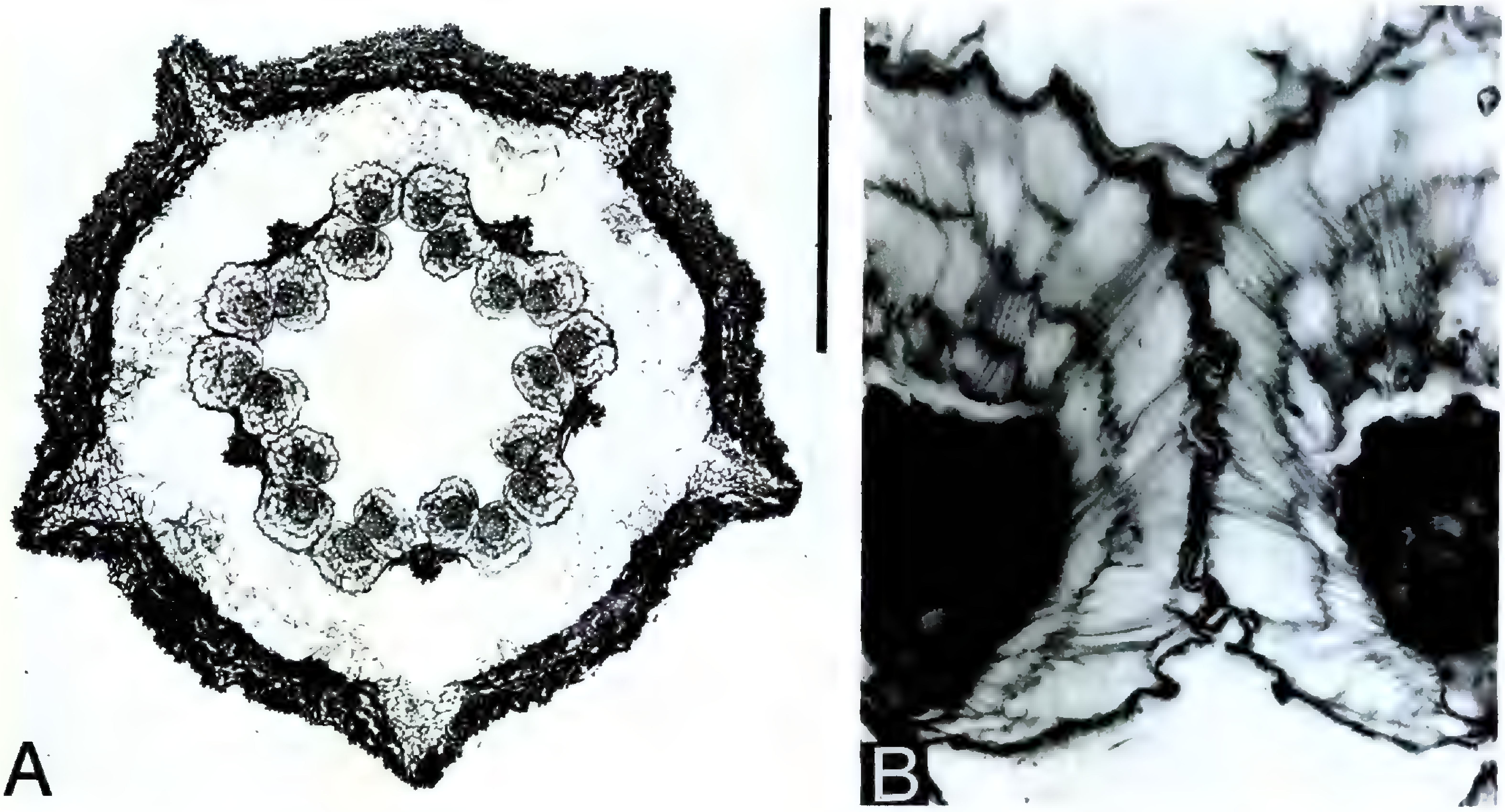

Figure 12. *Antherostele grandistipula.*—A. Cross section of corolla and anther tube.—B. Detail of A, showing agglutinate cuticles of pollen sacs from two neighboring anthers. Scale bar: 1 mm for A, 0.1 mm for B.

- All taxa of these two subgenera have a ringlike, nectar-producing disk, indicating nectar-collecting insects as pollinators. A disk, or nectar production for that matter, is absent in all other taxa of *Acranthera;* also cf. Bremekamp (1947).
- All representatives have flowers that are relatively much larger than in the other subgenera (corolla tubes ca. 20–40 mm long vs. hardly ever more than 10 mm), and the shape of the corolla tubes differs in being entirely infundibuliform or narrowly cylindrical below and infundibuliform above. Corolla colors—blue or violet in subgenus *Acranthera* and greenish to yellowish with pink spots inside in subgenus *Androtropis*—differ from those of the other subgenera.
- The filaments are very long (sometimes > 30 mm; several times longer than the anthers), whereas in other subgenera filament lengths rarely exceed 1 mm. In subgenus *Acranthera,* the filaments are free; in subgenus *Androtropis,* the lower half of the filaments is united into a filament tube which surrounds the style.

The two species Wong (1982) transferred from *Gardenia* to *Acranthera* belong to subgenus *Androtropis* (the distribution range of the subgenus is thus extended to include the Malay Peninsula). The illustration of one of them, *A. pulchella,* clearly shows the united filaments (Wong, 1982: fig. 3B), although this characteristic feature is not mentioned in the text.

Field observations of representatives of these taxa are needed.

ANTHEROSTELE (FIG. 12)

Bremekamp (1940) used the presence of an anther tube as one of the main characters separating *Antherostele* from *Urophyllum* and stressed that the former is the only genus in the *Urophyllum* alliance to show this phenomenon.

The genus has strictly cylindrical anther tubes. In contrast to, for example, *Argostemma* or *Steenisia* spp., apical connective appendages are not involved in the formation of the tubes. Minute apical connective appendages are present, but they—together with the very tips of the thecae—are curved outward. Except for the upper- and lowermost parts of the thecae, the anthers are united into a tube by agglutination of the cuticles of neighboring pollen sacs (Fig. 12).

This kind of anther tube formation is characteristic of the Asteraceae (Compositae; cf. Thiele, 1988). *Antherostele* appears to be the only rubiaceous genus in which the anther tube is formed this way.

From developmental studies of Asteraceae flowers it is known that the agglutination of the anthers occurs at a quite late stage of floral development (Thiele, 1988). This may also be the case in *Antherostele,* although developmental investigations could not be carried out (in available older bud

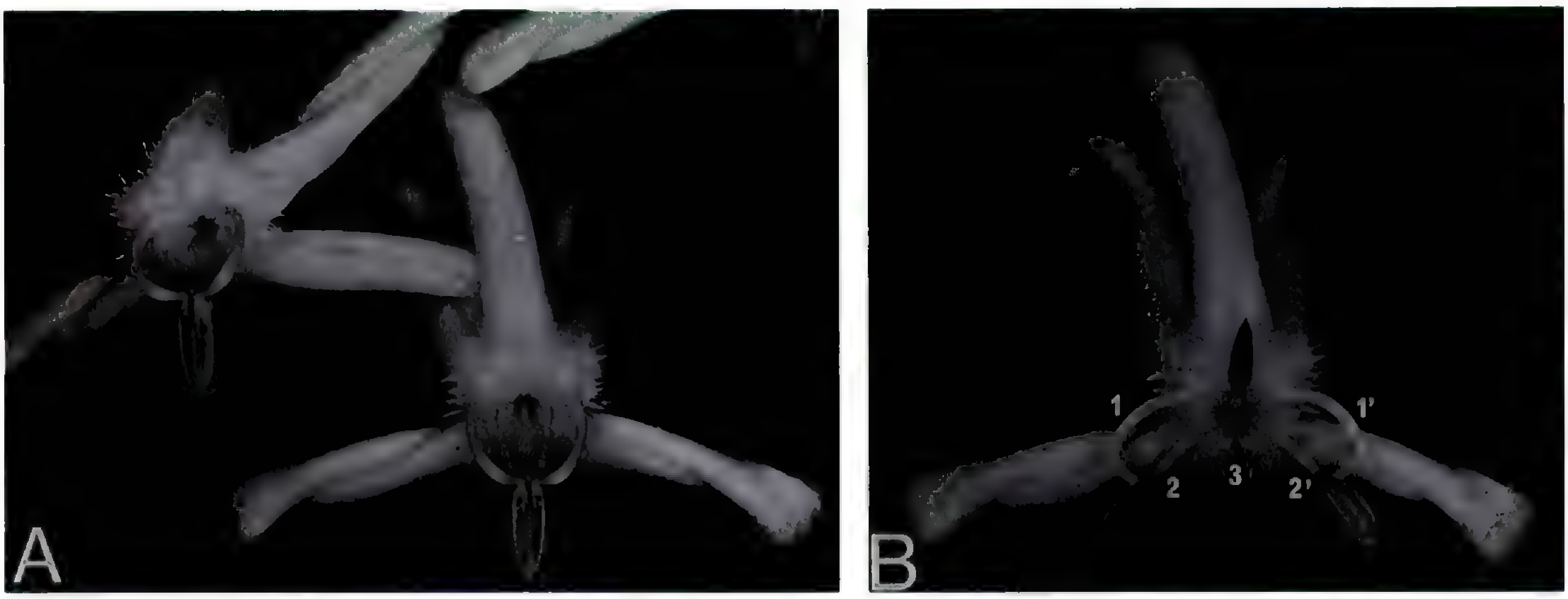

Figure 13. Flowers of *Posoqueria latifolia*.—A. Anthers still united.—B. Anthers separated: 2 pairs of lateral anthers (1 + 2; 1' + 2') and "lower" stamen (3) with its anther in an upper position (i.e., in front of the solitary, upper median corolla lobe; its filament blocks the entrance to the corolla tube).

stages, however, the anthers were already agglutinated).

The two species studied anatomically, *A. grandistipula* and *A. banahaensis*, show an identical situation. Judging from herbarium investigations, the two remaining species of the genus also do not differ in features of the anther tube (also cf. Bremekamp, 1940).

Comments on the pollination ecology.

Antherostele spp. are dioecious. The staminodes of female flowers, ca. half the size of the fertile stamens, are provided with completely sterile anthers which are also fused. In male flowers, the ovary is virtually absent, but a well-developed nonfunctional style is present. Whether the latter plays a secondary role in the pollination of *Antherostele* spp. is uncertain. It could serve as a receptacle for the pollen, which is released from the pollen sacs through inwardly directed longitudinal slits.

It is unlikely that in *Antherostele* the presence of an anther tube is linked with the occurrence of buzz pollination. Reasons for this tentative conclusion are:

- The genus's dioecy: Female flowers have relatively large, spreading stigma lobes that are exserted from the narrow corolla tubes and partially cover the corolla lobes. Because of the absence of the conspicuous stigma lobes, the appearance of the male flowers is different: the corolla lobes are fully exposed; the anther tubes are (except for the most apical parts) entirely included in the relatively long (normally > 10 mm) and narrow, cylindrical corolla tubes.
- The construction of the anther tubes themselves (absence of apical constrictions; hardly like anther cones with apical, porelike openings).
- The presence of a ringlike nectar-producing disk on the roof of the inferior ovary.
- The dense indumentum on the inside of the corolla, obscuring the entrance to the interior of the tube.

Actual field observations are needed.

POSOQUERIA (FIGS. 13, 14)

In a widely overlooked article, Müller (1866) presented detailed information on the remarkable "catapult mechanism" of the flowers of *P. latifolia* (as "*Martha* (*Posoqueria*?) *fragrans*") by which pollen is hurled at the pollinator. Later, Hallé (1967) dealt with the "pollen projection" in this genus, unknowingly dealing with the same species as Müller (because he considered "*Posoqueria fragrans*" to be specifically distinct from *P. latifolia*), and with *P. longiflora*.

When a flower of *P. latifolia* opens, all anthers are united into an ellipsoid structure (Fig. 13A), which is in an oblique position in relation to the corolla tube (a reflection of the distinctly zygomorphic bud shape). Normally, the anther complex points downward (as shown by Müller, 1866: fig. 2 and in fig. 13A), but it was observed that even within a single plant, the united anthers may point upward as the entire flower is turned 180°. This needs to be mentioned, because all of Hallé's (1967: pl. 40, 2–6) drawings of *P. latifolia* show flowers in this more atypical inverted position, i.e., the slightly enlarged "upper" corolla lobe (cf. Fig. 13A; comparable to the "standard" of a leguminous flower) is directed downward.

The anther head is held a few millimeters away from the throat of the corolla by the unequal filaments and, notwithstanding its "down-" or "upward" position, it leaves free access to the entrance of the corolla tube. As a pollinator (hawkmoths according to Müller, 1886; subsequently confirmed by other authors, e.g., Bawa & Beach, 1983: table 1) approaches, it will try to insert its proboscis into the corolla tube. By doing so, it is likely to knock against the filaments 1 or 1′ (cf. Fig. 13). Touching the curved part of the filaments will trigger the mechanism and cause the anther head to separate explosively and to ejaculate the pollen. Müller, studying flowers at his home, noted that even flies are able to trigger off the catapult mechanism.

The anther complex breaks when triggered into three parts: two lateral pairs of anthers (Fig. 13B, 1+2, 1′+2′), which move outward due to an abrupt and rapid movement of the filaments, and the fifth anther (labeled "3" in Fig. 13B). As the anther complex separates, the filament of this anther curves up- or downward (depending on the orientation of a flower; cf. above) so that its broadened base "blocks" or obscures the entrance to the corolla tube (cf. Fig. 13B, filament "3"). After a while (8 to 12 hours according to Müller), this filament starts straightening. Presumably due to shrinkage processes the filament eventually recurves, thus again "unblocking" the entrance to the corolla tube. At this stage, the flower is functionally female (the two stigma lobes, hidden in the corolla tube, have unfolded and presumably are receptive). Hallé's (1967) statement (repeated by Robbrecht, 1988: 122) that the anthers are initially being held together only by an intermingling of their hairs is incorrect.

A combination of microtome and SEM investigations revealed the following situation for *P. latifolia*:

All anthers have both apical and basal appendages (Fig. 14A). The appendages are, in contrast to the other examples presented in this paper, not formed by the connectives but by sterile "extensions" of the thecae. Figure 14C, a section through the basal part of an anther complex, documents that 5 × 2 appendages are present, whereby two appendages each (extensions of the thecae of a single anther) are associated with a given filament. The contact area between appendages from *neighboring* anthers is easily recognized by the intensively (dark) stained epidermal layers. As shown in Figure 14D, the epidermis layers are only united by a fusion of their cuticles. Already Müller (1866) had indicated that, under the microscope, the anthers might be united by means of special "kitt." In view of the later separation of the anther head into two anther pairs and a third anther, it should be noted that, at least in late bud stage, there appears to be no qualitative difference in the fusion of the five anthers. There is no obvious "stronger" fusion between the basal appendages of the stamens 1+1′ and 2+2′.

The pollen sacs dehisce longitudinally, and dehiscence takes place when the anther head is still intact (Fig. 14E). Consequently, an anther head in a stage as depicted in Figure 13A will contain a mass of pollen in its central hollow. The thecae of neighboring anthers themselves are not united (Fig. 14E). The close proximity of the pollen sac walls of adjacent anthers, nevertheless, effectively prevents pollen from reaching the exterior. The pollen grains, moreover, remain united in clumps (Fig. 14E; the presence of pollen kitt could, however, not be verified in the preserved material available). The later ejaculation of the pollen as "one spherical mass" (Hallé, 1967) is neither confirmed by personal observations by one of us (C. P.) nor by Müller (1866). The pollen is rather "shot" out of the exploding anther head in irregularly shaped clumps of different sizes (as is indicated by the section, Fig. 14E). Müller commented on the stickiness of these pollen clumps and noted they would even stick to smooth surfaces such as the blade of a knife.

Apically, each anther also has two sterile appendages, which are arranged on either side of the connective (Fig. 14F). Like the basal appendages, the apical ones, too, have an intensively stained epidermis, and in the most apical part the fused cuticles of neighboring epidermal layers ensure that the anthers are united (Fig. 14G). The area over which the apical appendages are united is very short. Less than half a millimeter from the very top, the appendages are already free. The section depicted in Figure 14F documents this. It is not at an entirely right angle to the anther head's long axis but slightly oblique. For this reason, only the apical appendages of the anther pair 1′+2′ are seen as being united.

The entire anther head, consequently, is a very "unstable" construction. Careful removal of the anther pair 1+2 from the anther complex depicted in Figure 14A, for example, showed that the apical appendage of anther 3 is hardly connected to the anther pair 1′+2′ (Fig. 14B; the arrows point to a distinct gap between the appendages). The fragile construction, however, is a prerequisite for the described catapult mechanism to work properly.

To date it is not known whether this unique pollen ejaculation mechanism occurs in all *Poso-*

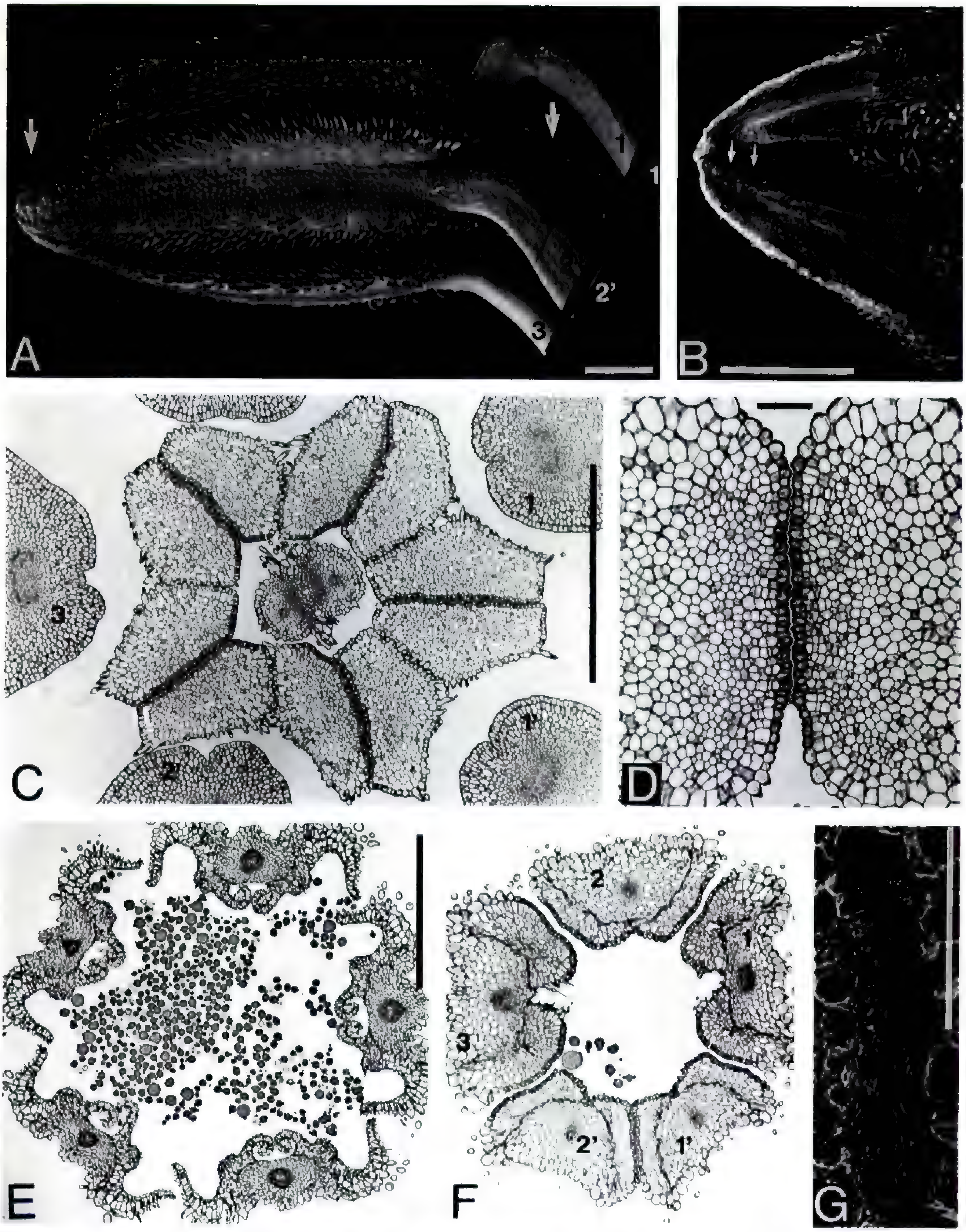

Figure 14. Androecium of *Posoqueria latifolia* (stamens numbered as in Figure 13B).—A. United anthers, removed from bud shortly before anthesis; note apical and basal appendages (arrows).—B. Apical anther appendages, stamens 1 + 2 removed; note loose connection between anther 3 and anthers 1′ + 2′ (arrows).—C. Cross section of basal part of anthers; from outside in, filaments (numbered as in A), ring of basal anther appendages (10 paired sterile extensions = basal parts of the thecae), and two-lobed stigma (from bud; at later developmental stages, anthers and stigmas will be widely separated).—D. Detail of C; contact zone between sterile basal theca appendages of two neighboring stamens.—E. Cross section through the middle of an anther complex (from a flower in a stage as depicted in Fig. 13A, i.e., anthers still held together by apical and basal appendages), showing longitudinally dehisced pollen sacs and pollen mass in the central hollow.—F. Cross section of uppermost part of apical anther appendages; note that each connective has remnants of sterile thecae on either side; only appendages 1′ + 2′ are in direct contact,

queria species. Burck (1884) dealt with a "*Posoqueria hirsuta*," which supposedly lacks this mechanism. The species is not included in Andersson's (1992) checklist of neotropical Rubiaceae, and Burck may have misidentified the plant investigated by him.

MISCELLANEOUS OTHER TAXA

Neurocalyx *Hook. (Argostemmateae)*.

The general floral morphology of this small genus (4 species; 3 in Sri Lanka, 1 in southern India) is similar to those *Argostemma* species with (sub)rotate corollas and anthers united into a tube. As in *Argostemma* spp., the androecium is comprised of introrsely dehiscent stamens with short, free filaments, and enlarged connectives are prominent on the outside of the anther tube; the pollen sacs open by means of longitudinal slits. Judging from Bremer's (1979) illustrations, agglutinated or fused apical connective appendages ensure the "stability" of the anther tube.

Corolla shape (starlike) and color (white; occasionally pink?) and yellow, exposed anther tube (cf. Bremer, 1984: fig. 1B, color illustration of *Neurocalyx gardneri*) suggest that *Neurocalyx*, from the context of pollination biology, is inseparable from numerous *Argostemma* species. The genus can safely be added to the group of buzz-pollinated Rubiaceae.

Siphonandrium *K. Schum. (incertae sedis)*.

This is a poorly known, dioecious New Guinean monotypic genus (*S. intricatum* K. Schum.) of uncertain taxonomic position (possibly belonging to the Morindeae s. str.; cf. Igersheim & Robbrecht, 1993). It appears to be unique in the Rubiaceae in that not only an anther but also a filament tube is present, i.e., a staminal tube. Quoting Schumann (1905), "The filaments are tightly fused and the anthers are glued together . . . so that one has the impression of only one anther being present." According to Schumann, the staminal tube (> 4 mm long) is exserted from the hypocrateriform corolla; the latter is characterized by a short tube (3 mm long) and relatively large, spreading lobes (5 mm long); no information on anther dehiscence is included. Based on these data, the possibility of buzz pollination cannot be excluded altogether.

No material was seen; verification is badly needed.

Molopanthera *Turcz. (Cinchoneae; provisionally transferred to Condamineeae by Andersson & Persson, 1991)*.

According to Schumann (1891: 10, 54), the androecium of this monotypic eastern Brazilian genus resembles that of *Posoqueria* "in all details." Like *Posoqueria*, it has zygomorphic flowers, and Schumann suggested that it "most probably" exhibits the same mechanism of explosive pollen release.

No material was seen; verification is needed.

Two more genera of the Condamineeae deserve mentioning: Tresanthera *Karst. and* Rustia *Klotzsch*.

In conjunction with buzz pollination, both genera are included in Buchmann's (1978) table 5. *Tresanthera* flowers have anthers in a tube- or conelike arrangement (whether actual agglutinations or fusions are present remains unknown). The apically inwardly curved anthers open by means of a subapical valvelike structure. Anther morphology seems to be comparable to that of *Argostemma neurocalyx* (cf. Fig. 6C) except that the opening mechanism of the pollen sacs is apparently more specialized. Floral structure definitely suggests the occurrence of buzz pollination.

In contrast, the anthers of *Rustia*, dehiscing with two apical pores, do not form an anther cone. The genus's flowers do not fall into the "*Solanum*-type" of pollen flowers category, although the occurrence of buzz pollination cannot be excluded altogether.

Actual field observations on taxa of both genera are encouraged.

Discussion

The investigations presented here reveal the diversity by which "synandry" (in a very wide sense) is achieved in genera and species of the Rubiaceae. It is remarkable that even within genera (*Argostemma*) there are considerable differences in detail (Table 1).

The occurrence of this phenomenon is scattered

←
as the section is slightly oblique rather than strictly perpendicular to the anther complex's long axis.—G. Contact zone between apical sterile theca appendages of two neighboring stamens from near the very apex of the anther complex. A, B, G: SEM-graphs; all others: LM-graphs. Scale bars: 1 mm (A; B; C = F; E); 0.1 mm (D; G).

Table 1. Summary of the androecium features, the type of anther dehiscence, and the presumed pollination mode of the investigated Rubiaceae.

Androecium	Anther dehiscence	Pollination	Taxa	Position
Anther tube present				
(1) Tube apically constricted, with porelike opening				
(1a) Apical connective appendages interlocked and agglutinated	longitudinal slits	buzz pollination	*Steenisia*	Rondeletieae
(1b) Apical connective appendages united, in part also pollen sac walls agglutinated	longitudinal slits	buzz pollination	*Argostemma* p.p.; *Neurocalyx* (?)	Argostemmateae
(2) Tube not apically constricted, strictly cylindrical; pollen sac walls agglutinated; connective appendages small, not united	longitudinal slits	?	*Antherostele*	Urophylleae
(3) Tube with partially united, crownlike apical connective appendages; anthers united by means of special cell layer	poricidal	buzz pollination	*Strumpfia*	incertae sedis
Anthers not united				
(1) Anthers long and narrow, connivent	poricidal	buzz pollination	*Argostemma* p.p. (e.g., *A. diversifolium*)	Argostemmateae
(2) Anthers flattened, in ± zygomorphic arrangement	poricidal	buzz pollination (?)	*Argostemma* p.p. (*A. neurocalyx*)	Argostemmateae
"Anther-style and stigma complex"				
Apical connective appendages and stigma apex united; in part formation of apical "windows" (= gaps between connective appendages)	longitudinal slits	buzz pollination (?)	*Acranthera* (p.p.)	Isertieae ?; incertae sedis
Anther head				
Basal and apical theca appendages united	longitudinal slits	"catapult mechanism"; hawkmoth	*Posoqueria* (also *Molopanthera* ?)	Gardenieae subtribe Gardeniinae (Condamineae)

and occasional in the family. With the exception of the undoubtedly closely allied species pair *Argostemma* and *Neurocalyx*, it does not seem to reflect relationships. Its presence must rather be seen as a pollination biological adaptation or specialization that has arisen repeatedly in the family.

Among the Rubiaceae with united anthers, the group of buzz-pollinated "*Solanum*-type" of pollen flowers is by far the most prominent.

In the Rubiaceae, a family in which the "basic" type of pollen sac dehiscence is by means of longitudinal slits (cf. Robbrecht, 1988: 86), the poricidally dehiscent anthers of buzz-pollination systems are mostly "replaced" by an alternative construction, i.e., solid anther tubes (with longitudinally dehiscent pollen sacs); see under *Argostemma* for details. Yet, some buzz-pollinated Rubiaceae have also evolved poricidally dehiscent buzz anthers (whereby this is not necessarily linked with the union of anthers into solid tubelike structures).

Argostemma is the only Rubiaceae genus known to date in which both anther tubes with longitudinally dehiscent pollen sacs and free anthers with apical pores are found. In addition to this "switch" to poricidal dehiscence, possibly indicating a more "advanced" state, *Argostemma* is also remarkable because of the repeated occurrence of zygomorphy both in androecium and corolla.

Apart from the very specialized, monotypic *Strumpfia* (cf. Igersheim, 1993a), other genera with anthers with apical pores are *Rustia*, *Tresanthera*, and *Stomandra*, all belonging to tribe Condamineeae. Detailed morphological and anatomical information on their stamens is, however, still lacking. At present it is not known whether the "apical pores" of their anthers are in fact homologous to "normal" apical pores as in, for example, *Argostemma diversifolium*, or are very specialized modifications.

The unique anther-style and stigma complexes of *Acranthera* spp. with their peculiar "windows" (gaps between the elongated apical connective appendages) may represent a hitherto totally unknown "alternative" construction of a buzz-pollinated blossom. Actual proof from field observations is lacking, however. In this context it must be stressed again that the amount of in-depth field studies on taxa of the Rubiaceae, in general, is dismally small. More emphasis on this kind of work is desperately needed.

Another example of the lack of field data and pollination ecological information is *Antherostele*. The morphological and anatomical evidence presented here, together with the investigation of herbarium material, does not provide adequate clues to the possible pollination mechanism. We are inclined to believe that it might not be buzz pollinated.

From the viewpoint of pollination ecology, *Posoqueria*—with its unique explosive pollen release mechanism—stands apart from the remainder of the investigated genera and species; its pollination by hawkmoths has been confirmed independently by several authors. The presented data, nevertheless, provide the anatomical explanation for the temporary union of their anthers and correct previously published information.

The pollination mechanism of the investigated *Posoqueria* spp. may or may not cast some doubt on the taxonomic position of the genus in the Gardenieae–Gardeniinae. The tribe is characterized by the presence of an "ixoroid mechanism," i.e., secondary pollen presentation by the style and/or outside of the stigma lobes (Robbrecht & Puff, 1986), but in the investigated *Posoqueria* spp. this feature is absent and, in fact, must be absent if the mechanism of pollen release is to function properly. The pollination biological specialization could be interpreted as being an aberrant feature, and the genus left in the Gardenieae. On the other hand, pollination mode, or the characteristics that go with it (e.g., zygomorphy of the corolla), could be taken as strong indications for a position outside the Gardenieae. Detailed investigations of additional character states should help in clarifying its position.

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SUBFAMILIAL AND TRIBAL RELATIONSHIPS IN THE RUBIACEAE BASED ON *rbc*L SEQUENCE DATA[1]

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Abstract

A parsimony analysis of *rbc*L sequences from 49 Rubiaceae genera (representing 23 tribes) and 7 outgroup taxa was performed. Species representing 48 genera of Rubiaceae were sequenced: *Anthospermum, Antirhea, Bertiera, Bouvardia, Calycophyllum, Catesbaea, Cephalanthus, Cinchona, Coffea, Cubanola, Deppea, Enterospermum, Erithalis, Exostema, Gardenia, Guettarda, Haldina, Hallea, Hamelia, Hillia, Hintonia, Hoffmannia, Hydnophytum, Ixora, Keetia, Luculia, Meyna, Mitriostigma, Morinda, Mussaenda, Nauclea, Nertera, Oldenlandia, Ophiorrhiza, Parapentas, Pentagonia, Pentas, Pentodon, Pinckneya, Pogonopus, Psychotria, Rachicallis, Rogiera, Rubia, Sarcocephalus, Theligonum, Uncaria*, and *Vangueria*. The cladistic analysis resulted in 24 equally parsimonious trees with a consistency index (C.I.) of 0.38. The results were analyzed to test higher-level classification and reconstruction of Rubiaceae phylogeny, and to place taxa with disputed taxonomic positions. There are three groups of taxa more or less corresponding to three of Robbrecht's four subfamilies: Cinchonoideae s. str., Ixoroideae s.l., and Rubioideae. There is no support for the subfamily Antirheoideae, the taxa of which are nested within Cinchonoideae s. str. and Ixoroideae s.l. The positions of *Luculia* and *Hintonia* are uncertain. The tribal positions (sensu Robbrecht) are supported for a majority of the genera, but other indicated relationships contradict earlier classification. The data, although based on a limited number of taxa, support the monophyly of the tribes Anthospermeae, Chiococceae s.l., Guettardeae, Hamelieae, Hedyotideae, Naucleeae s.l., Psychotrieae, and Vanguerieae, but there is no support for the positions and/or narrow circumscriptions of Cephalantheae and subtribe Mitragyninae (Cinchoneae), or for a wide circumscription of Cinchoneae. The *rbc*L data also give useful suggestions for taxonomic positions of the following genera with uncertain affiliations: *Bertiera, Catesbaea, Deppea, Hillia, Ophiorrhiza, Rachicallis*, and *Theligonum*.

The Rubiaceae are one of the most speciose families, especially in the tropics, with about 10,000 species (Mabberley, 1990). Biologically and morphologically they are diverse, with many different life forms and reproductive traits. The life forms vary from tiny herbs, epiphytes, lianas, and shrubs to tall trees, and the various kinds of flowers have different pollination systems, where pollen is spread directly from the stamens by insects, birds, or wind or secondarily from the upper part of the styles (stylar pollen presentation) by insects. There is a great variety of fruits and seeds dispersed by different agents, e.g., dry capsules with wind-dispersed seeds, dry dehiscent or indehiscent mericarps, or fleshy and animal-dispersed berries or drupes. The different fruit traits have been much used for higher-level classification. The Rubiaceae are known to be a family with difficult intrafamilial classification. A persistent problem with the different classification schemes proposed has been that one or a few characters have been used as absolute markers for the different taxonomic groups, and this has led to unnatural groupings in many cases.

Schumann (1891) divided the Rubiaceae into two subfamilies, Cinchonoideae and Coffeoideae, based on a single character, the number of ovules per locule. This character and Schumann's classification were almost totally rejected by later authors (Table 1 presents a comparison between tribal and subfamilial classification schemes historically used). Bremekamp (1954, 1966) instead emphasized testa structure, occurrence of albumin in the seeds, raphides, and the "ixoroid pollen presentation mechanism" (Ixoroideae), and he recognized eight subfamilies. Three of these subfamilies, the Cinchonoideae, the Rubioideae, and the Guettardoideae (= Antirheoideae), were accepted by Verdcourt (1958). He utilized the same characters as Bremekamp, but rejected the pollen presentation mechanism as a subfamilial character; instead, he

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Table 1. Previously proposed tribal and subfamilial classification schemes of all analyzed Rubiaceae genera compared to the results of the *rbc*L phylogeny. The tribes are indicated with the first three letters of the tribal names: ANThospermeae, CATesbaeeae, CEPhalantheae, CINchoneae, CHIococceae, COFfeeae, CONdamineeae, GARdenieae, GUEttardeae, HAMelieae, HEDyotideae, HILlieae, HIPpotieae, ISErtieae, MORindeae, NAUcleeae, OPHiorrhizeae, PAVetteae, PSYchotrieae, RONdeletieae, RUBieae, THEligoneae, VANguerieae; the subfamilies are indicated with the first four letters of the subfamilial names: ANTIrheoideae, CINChonoideae, COFFeoideae, HILLioideae, IXORoideae, RUBIoideae, UROPhylloideae.

Genera	Schumann (1891)	Verdcourt (1958)	Bremekamp (1966)	Robbrecht (1988)	*rbc*L-support[a]
Anthospermum	**ANT**-COFF	**ANT-RUBI**	=[b]	=	=
Antirhea	**GUE**-COFF	**GUE**-ANTI[c]	=	=	**GUE-CINC***
Bertiera	GAR-CINC	ISE[d]-RUBI	?-UROP	?	**?COF-IXOR***
Bouvardia	CIN-CINC	**HED-RUBI**	=	?[e]	**HED-RUBI**
Calycophyllum	CIN-CINC	=	—	=	?-**IXOR s.l.***
Catesbaea	GAR-**CINC**	CAT-**CINC**	—	CAT-?	**CHI-CINC***
Cephalanthus	**NAU-CINC**	=	?	CEP-ANTI	**NAU-CINC**
Chiococca	**CHI**-COFF	**CHI-CINC**	**CHI**-IXOR[f]	**CHI**-ANTI	**CHI-CINC**
Cinchona	**CIN-CINC**	=	=	=	=
Coffea	PAV[g]-CINC	PAV[g]-CINC	—	**COF-IXOR**	=
Cubanola	CON-**CINC**[h]	CON-**CINC**[h]	—	CON-**CINC**	**CHI-CINC**
Deppea	RON-**CINC**	?HED-RUBI	?	**HAM**-RUBI	**HAM-CINC***
Enterospermum	GAR-CINC	PAV[f]-CINC	**GAR-IXOR**	PAV-**IXOR**[i]	**GAR-IXOR**
Erithalis	**CHI**-COFF	—	—	**CHI**-ANTI	**CHI-CINC***
Exostema	CIN-**CINC**	—	—	=	**CHI-CINC***
Gardenia	**GAR**-CINC	=	**GAR-IXOR**	=	=
Guettarda	**GUE**-COFF	**GUE**-ANTI[c]	=	=	**GUE-CINC***
Haldina	**NAU-CINC**[j]	=[j]	CIN-**CINC**[j]	**NAU-CINC**	**NAU-CINC**
Hallea	**NAU-CINC**[k]	=[k]	CIN-**CINC**[k]	=[k]	**NAU-CINC**
Hamelia	GAR-**CINC**	**HAM**-RUBI	=	=	**HAM-CINC***
Hillia	CIN-**CINC**	?-RUBI	**HIL**-HILL	**HIL-CINC**	=
Hintonia	—	—	—	?	?
Hoffmannia	GAR-**CINC**	**HAM**-RUBI	=	=	**HAM-CINC***
Hydnophytum	**PSY**-COFF	**PSY-RUBI**	—	=	=
Ixora	PAV[g]-COFF	PAV[g]-CINC	—	PAV-IXOR	?-**IXOR**
Keetia	**VAN**-COFF[l]	**VAN**-CINC[l]	—	**VAN**-ANTI	**VAN-IXOR***
Luculia	CIN-CINC	—	—	=	?
Meyna	**VAN**-COFF[m]	**VAN**-CINC[m]	**VAN-IXOR**[m,n]	**VAN**-ANTI	**VAN-IXOR**
Mitriostigma	—	—	—	GAR-**IXOR**	?-**IXOR**
Morinda	**MOR**-COFF	**MOR-RUBI**	=[o]	=	=
Mussaenda	**ISE**[d]-CINC	=	=	=	**ISE-IXOR s.l.***
Nauclea	**NAU-CINC**	=	=	=	=
Nertera	**ANT**-COFF	**ANT-RUBI**	—	=	=
Oldenlandia	**HED**[p]-CINC	**HED-RUBI**	=	=	=
Ophiorrhiza	HED[k]-CINC	**OPH-RUBI**	**OPH**-UROP	**OPH-RUBI**	=
Parapentas	—	**HED-RUBI**	—	=	=
Pentagonia	ISE[d]-CINC	—	?	**HIP-?**	**HIP-IXOR s.l.***
Pentas	**HED**[p]-CINC	**HED-RUBI**	—	=	=
Pentodon	**HED**[p]-CINC	**HED-RUBI**	—	=	=
Pinckneya	CON-CINC	=	—	=	?-**IXOR s.l.***
Pogonopus	CON-CINC	—	—	=	?-**IXOR s.l.***
Psychotria	**PSY**-COFF	**PSY-RUBI**	=	=	=
Rachicallis	RON-CINC	—	—	HED-RUBI[q]	?-**CINC**
Rogiera	**RON-CINC**[r]	=[r]	=[r,s]	=[r]	=
Rubia	**RUB**[t]-COFF	**RUB-RUBI**	—	=	=
Sarcocephalus	**NAU-CINC**	**NAU-CINC**[v]	=[u]	=	=
Theligonum	—	—	—	**THE-RUBI**	=
Uncaria	**NAU-CINC**	=	CIN-**CINC**	=	**NAU-CINC**
Vangueria	**VAN**-COFF	**VAN**-CINC	**VAN-IXOR**[n]	**VAN**-ANTI	**VAN-IXOR**

emphasized external hair types. In the latest classification (Robbrecht, 1988), the Rubiaceae are divided into four subfamilies: Cinchonoideae, Ixoroideae, Rubioideae, and Antirheoideae (Table 1). Of these, the last subfamily differs distinctly from that of the earlier classifications, and includes more tribes than in Verdcourt's (1958) and Bremekamp's (1966) classifications. For example, in Antirheoideae Robbrecht included not only the tribe Guettardeae but also tribes formerly ascribed to Cinchonoideae (sensu Verdcourt, 1958; Cephalantheae, Retinophylleae, Alberteae), Ixoroideae (sensu Bremekamp, 1966; Chiococceae, Vanguerieae), and Rubioideae (sensu Bremekamp, 1966; Knoxieae, Craterospermeae). Most characters for Antirheoideae are variable, but generally taxa have solitary seeds with large embryos.

The use of single key characters in classification is usually very risky (e.g., the use of ovule number per locule, which formed the primary basis for the earlier classifications of the family) and will cause problems if the characters are in conflict; the tribe Vanguerieae has an ixoroid pollen presentation mechanism and fits well into Bremekamp's Ixoroideae, but also has solitary seeds with large embryos that make it a suitable member of Robbrecht's Antirheoideae. Similar problems have also emerged for particular genera, e.g., *Bertiera*, *Catesbaea*, *Deppea*, *Hillia*, *Ophiorrhiza*, *Pentagonia*, *Rachicallis*, and *Theligonum*, which are known to be difficult to classify due to lack or contradiction of certain characters. In such a large family as Rubiaceae it is easy to imagine, and it has also been shown (Eriksson & Bremer, 1991; Bremer & Eriksson, 1992), that characters have evolved and/or been lost repeatedly during evolution. Hence, we cannot use absolute markers, or "cardinal characters," for circumscription of taxa. We must consider all characters, not least the molecular, and in the most parsimonious way attempt to identify monophyletic groups that can be circumscribed as taxonomic entities. Currently we do not have the information from all genera of the family to perform a complete analysis of the family based on "all characters," but we can test and search for informative characters that later on can be combined in a large parsimony analysis.

In the past couple of years the ribulose-1,5-bisphosphate carboxylase (*rbc*L) gene of the chloroplast genome has been much used for analyses of angiosperm phylogeny (e.g., Chase et al., 1993; Olmstead et al., 1993), and it has previously been shown to be very useful for family-level analysis (Doebley et al., 1990; Soltis et al., 1990; Conti et al., 1993). The strength of molecular data is that they are independent of morphological characters, and we can discover relationships that might be hidden if only morphological characters are inves-

←

[a] Tribal and subfamilial placement supported by the *rbc*L data and all classifications in the different schemes supported by the *rbc*L data are in boldface.
[b] "=" Denotes the same tribe and subfamily as previous author that has considered the particular genus.
"*" Indicates placement supported by *rbc*L data but not proposed in Schumann (1891), Verdcourt (1958), Bremekamp (1966), or Robbrecht (1988).
"—" Denotes a genus (or a commonly used synonym) not considered by the author, "?" denotes uncertain position according to the author.
[c] Under the synonym Guettardoideae.
[d] Under the synonym Mussaendeae.
[e] Uncertain position CIN-CINC or HED-RUBI.
[f] The genus *Chiococca* is not mentioned but the tribe Chiococceae is.
[g] Under the synonym Ixoreae.
[h] Earlier included in the genus *Portlandia*.
[i] Included in the genus *Tarenna*.
[j] Earlier included in the treated genus *Adina*.
[k] Earlier included in the genus *Mitragyna*.
[l] Earlier included in the genus *Canthium*.
[m] Earlier included in the genera *Vangueria* or *Canthium*.
[n] The genus *Vangueria* (*Meyna*) is not mentioned but the tribe Vanguerieae is.
[o] The genus *Morinda* is not mentioned but the tribe Morindeae is.
[p] Under the synonym Oldenlandieae.
[q] In index and classification indicated as HED but in text on p. 39 as RON.
[r] Earlier included in the genus *Rondeletia*.
[s] The genus *Rondeletia* (*Rogiera*) is not mentioned but the tribe Rondeletieae is.
[t] Under the synonym Galieae.
[u] Earlier included in the genus *Nauclea*.

Table 2. Sources of living material extracted for DNA. Species names are followed by a four- or three-letter suffix. In the outgroup taxa the suffix indicates the first four letters of the family names: APOCynaceae, GENTianaceae, and OLEAceae according to Mabberley (1990); GELSemiaceae, SPIGeliaceae, STRYchnaceae sensu Struwe et al. (1995). The listed subfamilies of Rubiaceae follow Robbrecht (1988), as do the tribal positions (indicated with a three-letter suffix; cf. Table 1) for most taxa; however, * indicates tribal positions according to Bremer (1992).

Species	Source/voucher information[a]	New *rbc*L sequences reported in this paper will appear in the EMBL database under the accession number
Outgroup Taxa		
*Anthocleista grandiflora*GENT	Olmstead et al., 1993	
*Exacum affine*GENT	Olmstead et al., 1992	
*Kopsia fruticosa*APOC	Olmstead et al., 1993	
*Ligustrum vulgare*OLEA	Olmstead et al., 1992	
*Mostuea brunonis*GELS	Olmstead et al., 1993	
*Spigelia marilandica*SPIG	Donoghue et al., 1992	
*Strychnos nux-vomica*STRY	Olmstead et al., 1993	
Rubiaceae		
Subfamily Antirheoideae		
*Antirhea lucida*GUE	Bremer & Jansen, 1991	x83624
*Cephalanthus occidentalis*CEP	Bremer & Jansen, 1991	x83629
*Chiococca alba*CHI	Olmstead et al., 1993	
*Hintonia latiflora*CHI*[b]	Bremer & Jansen, 1991	x83643
*Cubanola dominguensis*CHI*	JBSD, McDowell 4472 (Duke)	x83632
*Erithalis fruticosa*CHI	Bremer & Jansen, 1991	x83635
*Exostema caribaeum*CHI*	Bremer & Jansen, 1991	x83636
*Guettarda uruguensis*GUE	Bremer & Jansen, 1991	x83638
*Keetia zanzibarica*VAN	Tanzania, Bremer 3069 (UPS)	x83647
*Meyna tetraphylla*VAN	Tanzania, Bremer 3074 (UPS)	x83649
*Vangueria madagascariensis*VAN	Bremer & Jansen, 1991	x83670
Subfamily Cinchonoideae		
*Calycophyllum candidissimum*CIN	Bremer & Jansen, 1991	x83627
*Cinchona succirubra*CIN	Bremer & Jansen, 1991	x83630
*Haldina cordifolia*NAU	Bremer & Jansen, 1991	x83639
*Hallea (Mitragyna) rubrostipulata*CIN	BR 83-1132, Robbrecht s.n. (UPS)	x83640
*Hillia triflora*HIL[c]	COLO, Bremer 3101 (UPS)	x83642
*Luculia grandifolia*CIN	Bremer & Jansen, 1991	x83648
*Mussaenda erythrophylla*ISE	Bremer & Jansen, 1991	x83652
*Nauclea orientalis*NAU	C, Bremer 3001 (UPS)	x83653
*Pinckneya pubens*CON	Bremer & Jansen, 1991	x83661
*Pogonopus speciosus*CON	Bremer & Jansen, 1991	x83662
*Rogiera suffrutescens*RON	Bremer & Jansen, 1991	x83665
*Sarcocephalus latifolius*NAU	P, Bremer 2726 (UPS)	x83667
*Uncaria rhyncophylla*CIN	UPS, no voucher	x83669
Subfamily Ixoroideae		
*Coffea arabica*COF	Bremer & Jansen, 1991	x83631
*Enterospermum coriaceum*PAV	Bremer & Jansen, 1991	x83634
*Gardenia thunbergia*GAR	Bremer & Jansen, 1991	x83637
*Ixora coccinea*PAV	CONN, Birgitta Bremer 2719 (UPS)	x83646
*Mitriostigma axillare*GAR	Bremer & Jansen, 1991	x83650
Subfamily Rubioideae		
*Anthospermum herbaceum*ANT	Tanzania, Bremer 3093 (UPS)	x83623
*Deppea grandiflora*HAM	P, Bremer 2724 (UPS)	x83633
*Hamelia cuprea*HAM	Bremer & Jansen, 1991	x83641

Table 2. Continued.

Species	Source/voucher information[a]	New *rbc*L sequences reported in this paper will appear in the EMBL database under the accession number
Hoffmannia refulgens × *ghiesbreghtii*HAM	Bremer & Jansen, 1991	x83644
*Hydnophytum formicarum*PSY	Bremer & Jansen, 1991	x83645
*Morinda citrifolia*MOR	UPS, Bremer 3106 (UPS)	x83651
*Nertera granadensis*ANT	Bremer & Jansen, 1991	x83654
*Oldenlandia cf. corymbosa*HED	Tanzania, Bremer 3075 (UPS)	x83655
*Ophiorrhiza mungos*OPH	BR71-1493; Robbrecht s.n. (UPS)	x83656
*Parapentas silvatica*HED	Tanzania, Bremer 3091 (UPS)	x83657
*Pentas lanceolata*HED	Bremer & Jansen, 1991	x83659
*Pentodon pentandrus*HED	Tanzania, Bremer 3082 (UPS)	x83660
Psychotria kirkii (bacteriophila) PSY	Bremer & Jansen, 1991	x83663
*Rachicallis americana*HED	FTG 64-266, Fanning KF81	x83664
*Rubia tinctorum*RUB	UPS, Bremer 3300 (UPS)	x83666
*Theligonum cynocrambe*THE	UPS, Struwe 1004 (UPS)	x83668
Incertae sedis		
*Bouvardia glaberrima*CIN/HED	Bremer & Jansen, 1991	x83626
*Bertiera breviflora*INC	WAG (Cameroun), Setten 713 (WAG)	x83625
*Catesbaea spinosa*CAT	Bremer & Jansen, 1991	x83628
*Pentagonia macrophylla*HIP	DUKE, McDade 595A	x83658

[a] Reference to literature if the specimen has been used before. For the other taxa the source of the living material is first indicated followed by the collector of the voucher and the herbarium in which it is deposited: BR, C, COLO, CONN, DUKE, FTG, JBSD, P, UPS, WAG.

[b] Charlotte Taylor has kindly informed us that this species erroneously was indicated as *Coutarea latiflora* in Bremer & Jansen (1991).

[c] The investigated species *Hillia triflora* was described as a *Ravnia* but in a recent revision (Taylor, 1989) that genus is now considered to be a subgenus within *Hillia*.

tigated. In this study we have sequenced the *rbc*L gene of 48 different Rubiaceae species from genera representing 23 tribes and 4 subfamilies. The main aim was to see if the nucleotide variation in the *rbc*L gene was enough to answer the following: (1) Is *rbc*L a useful character set for phylogenetic analysis within the Rubiaceae? (2) Can we use the *rbc*L results to test higher-level classification in the family? To what extent is the result contradicting or corresponding to the classification, or to the earlier phylogenetic analyses? (3) Are analyses of *rbc*L sequences useful to pinpoint taxonomic positions of disputed genera (e.g., *Bertiera*, *Catesbaea*, *Deppea*, *Hillia*, *Ophiorrhiza*, *Pentagonia*, *Rachicallis*, and *Theligonum*)?

Material and Methods

The *rbc*L gene has been analyzed from 56 species representing 7 outgroup genera and 49 Rubiaceae genera. Of these, 48 sequences are new and the sequences are accessioned in the EMBL database as x83623–x83670 (Table 2). The remaining eight sequences included in the analyses are from Genbank (Table 2). The sampling of Rubiaceae was done to cover as many tribes as possible and represents 23 tribes and 4 subfamilies (according to the classification of Robbrecht, 1988).

Total DNA was extracted (Saghai-Maroof et al., 1984; Doyle & Doyle, 1987) from fresh or silica gel dried (Chase & Hills, 1991) leaves. Double-stranded DNA of the *rbc*L gene was amplified by PCR with two synthetic primers (Olmstead et al., 1992): the 5′ primer was identical to the first 26 nucleotides of *rbc*L of tobacco, and the 3′ primer corresponds to a region ca. 100 nucleotides downstream from the coding region. This covers the entire coding gene excluding the first 26 nucleotides. A second run with asymmetric amplification was performed to get single-stranded DNA (Kaltenboeck et al., 1992). Single-stranded DNA was sequenced using internal primers designed by G. Zurawski (DNAX Research Institute).

The data matrices in the phylogenetic analyses

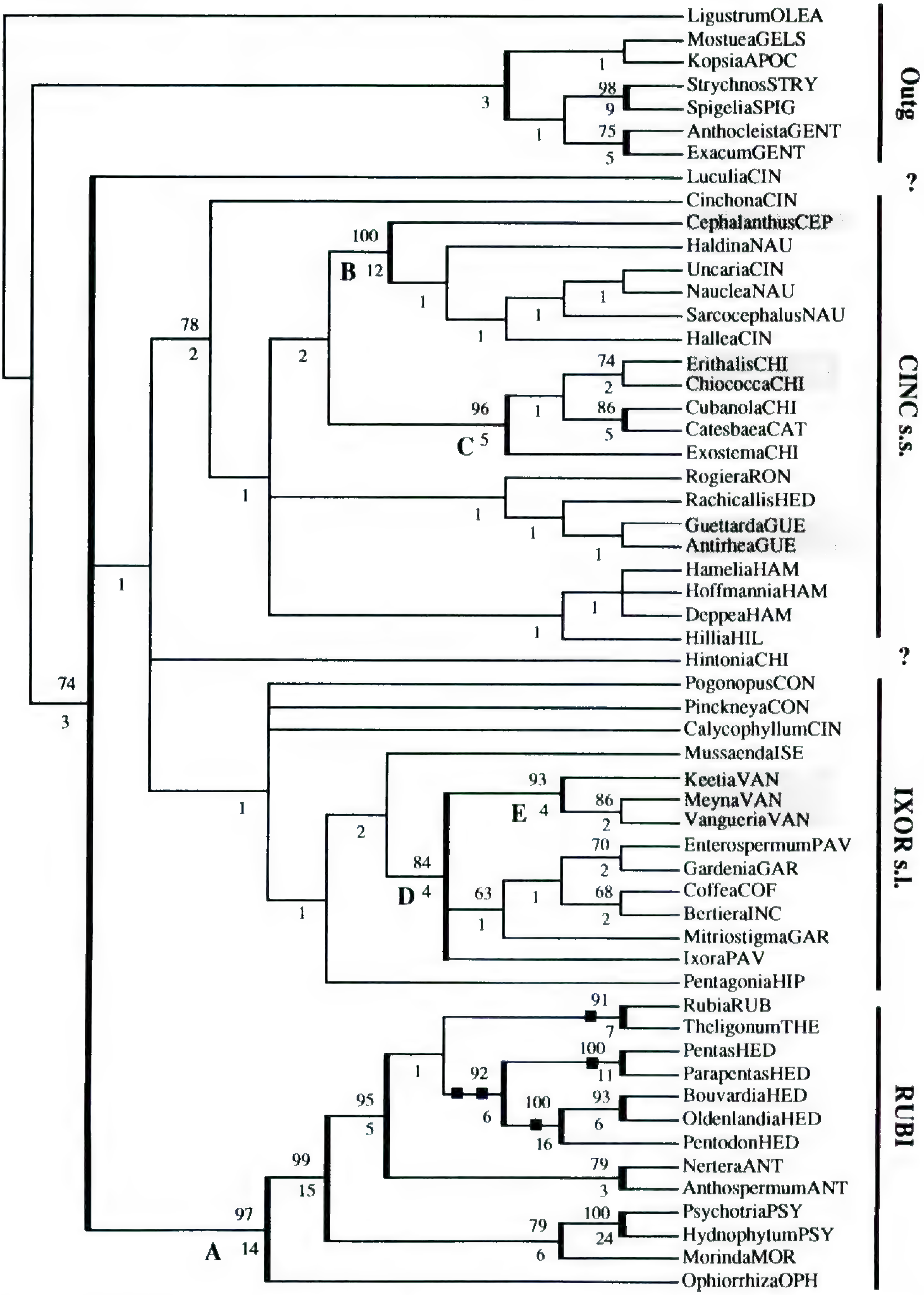

Figure 1. Strict consensus tree of 24 equally parsimonious trees of Rubiaceae based on *rbc*L sequences. The rooting of the tree was accomplished by making the "outgroup paraphyletic with respect to the ingroup" (an option in PAUP). Families are indicated by a four-letter suffix corresponding to the families in Table 2, and tribal positions are indicated by a three-letter suffix corresponding to the tribes in Table 2. Vertical bars and corresponding letters

comprise characters corresponding to each nucleotide position (27 to 1428) of the *rbc*L sequence. Only phylogenetically informative characters (295 positions) were analyzed. Parsimony analyses were conducted using PAUP version 3.1.1 (Swofford, 1991) on a MacQuadra840, under the assumptions of Fitch parsimony (Fitch, 1971). We searched with heuristic methods, with random addition of sequences and 100 replications, and TBR branch swapping. To evaluate the branch support (Fig. 1) we have performed a Bremer support analysis (Bremer, 1988; Källersjö et al., 1992), as well as a bootstrap analysis with 1000 replicates (Felsenstein, 1985).

The outgroups were chosen in agreement with results from earlier investigations; six taxa were chosen from other families of the Gentianales (*Kopsia*—Apocynaceae, *Mostuea*—Gelsemiaceae, *Strychnos*—Strychnaceae, *Spigelia*—Spigeliaceae, and *Anthocleista* and *Exacum*—Gentianaceae) and one taxon *Ligustrum* (Oleaceae) was selected from outside this supposed monophyletic order. The position of Rubiaceae in the order Gentianales was first suggested by Utzschneider (1947) and later established by Wagenitz (1959, 1964). This position is accepted by most systematists dealing with higher-level classification (Dahlgren, 1980; Thorne, 1983, 1992; Takhtajan, 1987) and is also supported by molecular and morphological data (Downie & Palmer, 1992; Bremer & Struwe, 1992; Chase et al., 1993; Olmstead et al., 1993; Bremer et al., 1994; Struwe et al., in press).

Results

In the analysis (Figs. 1 and 2), of the 56 taxa 456 nucleotide positions were variable and of these 295 were phylogenetically informative. Among the variable characters 109 (24%) are first positions, 83 (18%) are second positions, and 264 (58%) are third positions. Of all the nucleotide substitutions only a few resulted in synapomorphic and non-homoplastic changes in amino acid composition. These five unique changes in amino acids occur in subfamily Rubioideae. *Rubia* and *Theligonum* share a change from 2Serine to Glycine (nucleotides 28–30). All taxa of the Hedyotideae share the changes from Threonine to Asparagine (nucleotides 736–738), and the change from Leucine to Methionine (nucleotides 1111–1113). Within the Hedyotideae *Pentas* and *Parapentas* share a change from Valine to 2Serine (nucleotides 469–471). The last unique change occurs in nucleotide positions 1315–1317; these positions are very variable but *Bouvardia, Oldenlandia, and Pentodon* are the only taxa with 1Serine in these positions.

The heuristic search with 100 random additions of sequences including only phylogenetically informative characters resulted in 24 equally parsimonious trees 1033 steps long (395 minimal possible steps) with a C.I. of 0.38 and a retention index (R.I.) of 0.66.

All the equally parsimonious trees are very similar concerning positions of most taxa. The Rubiaceae are monophyletic and the sister group to the rest of the Gentianales (Figs. 1 and 2). In all the 24 trees (Fig. 1 = the strict consensus tree of these 24 trees) there are three large clades (CINC s.s., IXOR s.l., and RUBI, which can be seen in the strict consensus tree). These groups of taxa more or less correspond to three of the four subfamilies of the Rubiaceae (Robbrecht, 1982, 1988), namely the Cinchonoideae, the Ixoroideae, and the Rubioideae, although the circumscriptions differ from Robbrecht's assessment. The fourth subfamily, Antirheoideae, receives no support from this analysis. Furthermore, there are two genera (*Luculia* of the tribe Cinchoneae and *Hintonia* of uncertain position) whose positions differ distinctly in the different trees. *Luculia* is positioned at the base of the whole CINC s.s./IXOR s.l. clade or at the base of the RUBI clade, and *Hintonia* is positioned at the base of the IXOR s.l. clade or at the base of the CINC s.s. clade. The most stable, or most supported, part of the trees corresponds to the subfamily Rubioideae (RUBI, Fig. 1: A) where almost all nodes remain in trees two steps longer (Fig. 1; indicated with heavy bars). Other well-supported clades include the tribe Naucleeae s.l. (Fig. 1: B), the Chiococceae s.l., including *Cubanola/Catesbaea* (Fig. 1: C), Ixoroideae s. str./Van-

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represent: Outg = outgroups, CINC s.s. = subfamily Cinchonoideae s. str., IXOR s.l. = subfamily Ixoroideae s.l., RUBI = subfamily Rubioideae. Question marks indicate taxa with uncertain position. Shaded genera are members of Robbrecht's (1988) subfamily Antirheoideae. Numbers below branches indicate number of extra steps required to collapse the particular node. Nodes with heavy bars require more than 2 extra steps to collapse. Numbers above nodes indicate bootstrap fractions above 60%. Black squares indicate synapomorphic and non-homoplastic changes in amino acid composition (C.I. = 1.0). Clade A = subfamily Rubioideae, clade B = tribe Naucleeae s.l., clade C = tribe Chiococceae s.l., clade D = Ixoroideae s. str./Vanguerieae, clade E = tribe Vanguerieae.

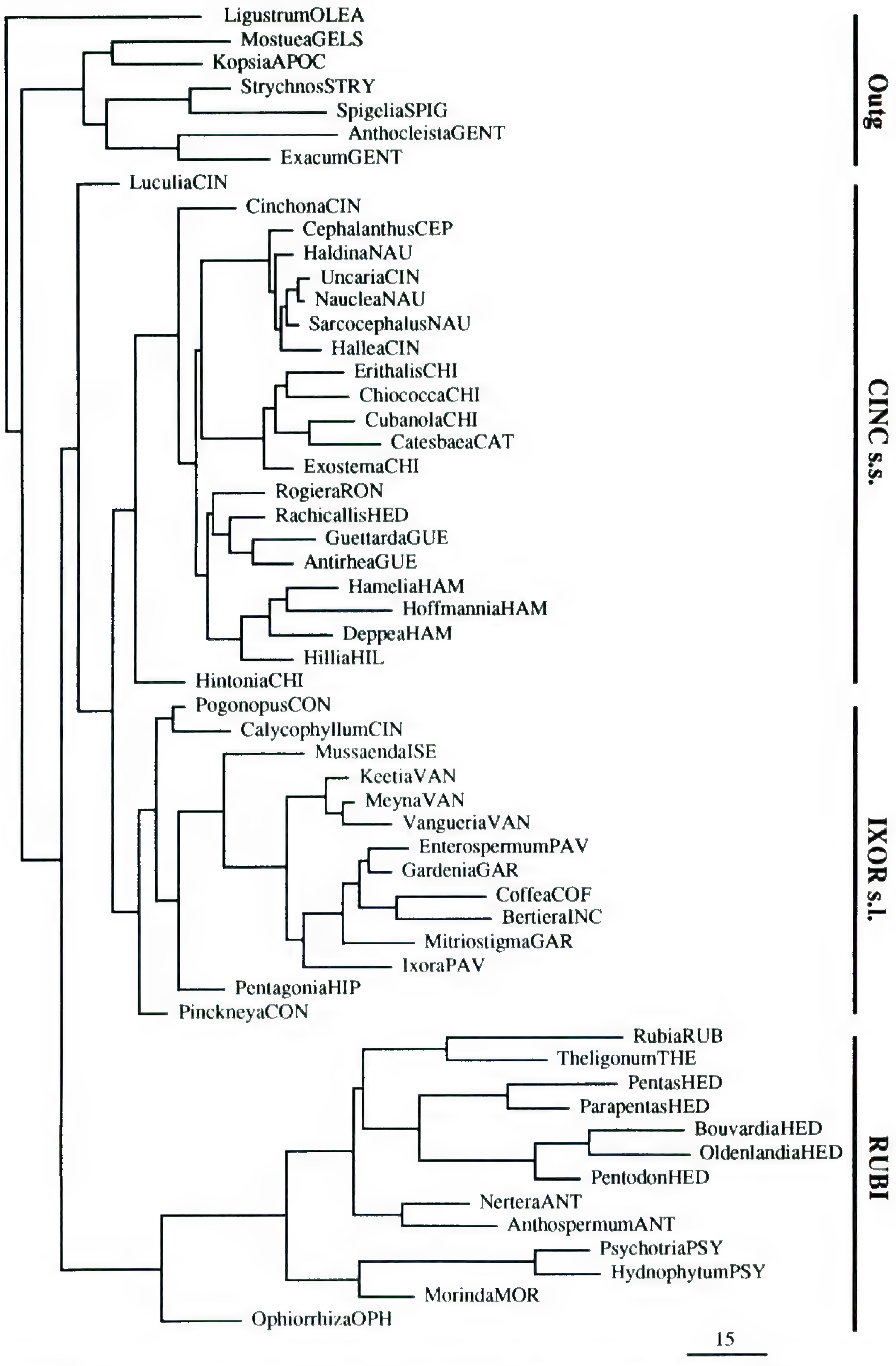

Figure 2. Sample phylogram, one of 24 equally parsimonious trees of Rubiaceae based on *rbc*L sequences, 1033 steps long with a C.I. of 0.38 and an R.I. of 0.66 (only phylogenetically informative characters are included). The rooting of the tree was accomplished by making the "outgroup paraphyletic with respect to the ingroup" (an option

guerieae (Fig. 1: D), and the Vanguerieae (Fig. 1: E).

The Rubioideae (RUBI) clade is supported by 18 characters (Fig. 2) and is one of the strongest in the analysis; the tree topology for this group is the same in all trees. *Ophiorrhiza* is the most basal taxon, followed by the Psychotrieae/Morindeae clade, which is the sister group to the remaining Rubioideae: the Anthospermeae, Hedyotideae, Theligoneae, and Rubieae. All taxa of the Rubioideae clade belong to the subfamily Rubioideae (sensu Robbrecht, 1988).

The IXOR s.l. clade includes all taxa of the Ixoroideae s. str. (Robbrecht, 1988), namely the members of the tribes Gardenieae, Pavetteae, and Coffeeae, but also the Vanguerieae (subfamily Antirheoideae, sensu Robbrecht) and some genera usually included in the Cinchonoideae: *Mussaenda* (Iserteae), *Calycophyllum* (Cinchoneae), and *Pinckneya* and *Pogonopus* (Condamineeae). The Vanguerieae/Ixoroideae s. str. clade (Fig. 1: D) is well supported and remains in trees four steps longer than the most parsimonious trees.

The CINC s.s. clade corresponds to parts of the Cinchonoideae, namely the tribes Cinchoneae s. str., Naucleeae s.l., Chiococceae s.l., Rondeletieae, Hillieae, but also Hamelieae, which usually is placed in the Rubioideae (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988), and the Guettardeae, which used to be accepted as, or a part of, the subfamily Antirheoideae (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988).

Of the taxa with disputed taxonomic positions, *Theligonum* is in this analysis the sister taxon to *Rubia* within the Rubioideae, *Ophiorrhiza* is sister taxon to all other Rubioideae, *Bertiera* is nested within Ixoroideae s. str., *Pentagonia* is more basal in that same clade, *Hillia* is close to Hamelieae in the Cinchonoideae, *Catesbaea* is close to *Cubanola* in the Chiococceae s. str. (Cinchonoideae), and *Rachicallis* belongs to Cinchonoideae.

Discussion

The present analysis demonstrates that *rbc*L data are informative for phylogenetic studies within Rubiaceae. The resolution of the strict consensus tree is high, with most branches resolved (35 of 41 within Rubiaceae), and thus most taxa have the same position in all trees. Compared to *rbc*L analyses of other angiosperm families, e.g., Onagraceae (Conti et al., 1993) and Asteraceae (Kim et al., 1992), our analysis results in more trees (24 compared to 1 and 8, respectively) and a lower consistency index (0.38 compared to 0.63 and 0.47, respectively). However, in these studies many fewer taxa (12 and 28 compared to 56) were analyzed, and at least the consistency index is sensitive to the number of taxa (Sanderson & Donoghue, 1989). If we compare the present analysis with similar phylogenetic analyses of Rubiaceae, the resolution of the consensus trees and the consistency indices are about the same as in the restriction site analysis (Bremer & Jansen, 1991) and the morphological analyses (Bremer & Struwe, 1992). The stability in terms of branch support is high but varies between the different branches (Fig. 1).

The answer to the second question, whether we can use *rbc*L to test higher-level classification within Rubiaceae, is yes. With this restricted study, including only about 8% of all the genera of the family, we can test the alleged monophyly of 12 tribes, and also the relationships postulated between these. In a comparison with the latest classification of Rubiaceae (Robbrecht, 1988), we find that our data support the tribal classification for 65% of the genera, and for about 16% the results are contradictory (Table 1); for the remaining part the *rbc*L data are not informative due to incomplete sampling of taxa or homoplasy in data.

The phylogeny and the subfamilial classification are more problematic. The *rbc*L phylogeny shows three basal groups of taxa, more or less corresponding to three of the four subfamilies (Robbrecht, 1988), Cinchonoideae s. str., Ixoroideae s.l., and Rubioideae (Figs. 1 and 2). The fourth subfamily, Antirheoideae, is scattered within the Cinchonoideae s. str. and Ixoroideae s.l. and thus is not supported. Of the three clades, the Rubioideae are the most stable part of the tree, supported by 18 characters, and all branches except one remain in trees three steps longer. The branch support for the other two basal clades (CINC s.s. and IXOR s.l.) is much weaker and for some investigated taxa (*Luculia* and *Hintonia*) the position is equivocal. However, within the two clades some

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in PAUP). Families are indicated by a four-letter suffix corresponding to the families in Table 2, and tribal positions are indicated by a three-letter suffix corresponding to the tribes in Table 2. Vertical bars and corresponding letters represent: Outg = outgroups, CINC s.s. = subfamily Cinchonoideae s. str., IXOR s.l. = subfamily Ixoroideae s.l., RUBI = subfamily Rubioideae. Branch lengths are proportional to the number of substitutions supporting a node; scale bar equals 15 substitutions.

nodes are stable and strengthen various relationships. The most stable nodes correspond to Naucleeae s.l. (Fig. 1: B), Chiococceae s.l. (Fig. 1: C), Vanguerieae (Fig. 1: E), and Ixoroideae s. str./Vanguerieae (Fig. 1: D). It is obvious that the molecular data in many parts are congruent with earlier classifications or phylogenetic analyses, but in other parts there are contradictions. In the following we will discuss each of the major groups of the Rubiaceae supported by our analysis.

THE CINCHONOIDEAE S. STR. (CINC S.S.) CLADE (Figs. 1 and 2)

The Cinchonoideae s. str. clade includes a majority of the Cinchonoideae, but not the genera *Pogonopus*, *Pinckneya*, *Calycophyllum*, and *Mussaenda*. Nested within the Cinchonoideae clade are parts of Antirheoideae (Robbrecht, 1988), and thus Antirheoideae is polyphyletic. Some of Robbrecht's (1988) Cinchonoideae tribes are supported by our data. An interesting exception is that the tribe Cinchoneae sensu Robbrecht is not supported. We studied *Cinchona* and five other genera of Cinchoneae (*Calycophyllum*, *Exostema*, *Hallea*, *Luculia*, and *Nauclea)*, but none of these five genera came out closely related to *Cinchona*, a result in agreement with a cladistic analysis of Cinchonoideae by Andersson & Persson (1991). Two of the genera we studied, *Uncaria* and *Hallea*, were moved to a tribe Coptosapelteae by Andersson & Persson (1991). However, there was no cladistic support for that grouping in their data, and their tribe is more an example of lumping all paleotropical taxa that earlier were part of the Cinchoneae. Our data definitely point to a position of *Uncaria* and *Hallea* within the tribe Naucleeae, where they were placed earlier (Verdcourt, 1958). The position within the Naucleeae is well supported by morphological data (e.g., capituliform inflorescences, and pollen presentation from the "soon-to-be-receptive stigmatic surface"; Imbert & Richards, 1993). *Exostema* belongs to the Chiococceae s.l. and *Calycophyllum* belongs to the Ixoroideae s.l. clade, a position also supported by restriction site data (Bremer & Jansen, 1991).

Hintonia has an uncertain position at the base of the Cinchonoideae clade or at the base of the Ixoroideae s.l. clade. *Luculia* has an even more uncertain position either at the base of Cinchonoideae s. str., or at the base of Cinchonoideae s. str./Ixoroideae s.l., or at the base of the Rubioideae. Based on restriction site data *Luculia* is placed at the base of the family (Bremer & Jansen, 1991); however, if that tree is rerooted with the root inbetween the Rubioideae and the rest of the family, the genus becomes closely related to *Cinchona* and *Rogiera*; based on morphological data the genus is placed at the base of the family (Bremer, 1992; Bremer & Struwe, 1992). We have at the moment no suggestions for a tribal position for *Luculia*. Andersson & Persson (1991) moved *Luculia*, together with other paleotropical taxa, to the Coptosapelteae, but according to our results *Luculia* is at least not close to *Uncaria* and *Hallea*.

The tribe Naucleeae has in recent years been split into different tribes and subfamilies (Bremekamp, 1966; Ridsdale, 1975, 1976, 1978; Robbrecht, 1988), but our results support a broad circumscription of the tribe in accordance with Verdcourt's (1958) classification. *Hallea* (*Mitragyna*) and *Uncaria* are united into subtribe Mitragyninae of the Cinchoneae in Robbrecht's (1988) classification. Such a grouping is paraphyletic according to our results (Figs. 1 and 2). *Cephalanthus* is placed in a different subfamily (Antirheoideae) by Robbrecht (1988), but our analysis shows it to be very close to the other members of the Naucleeae (Figs. 1 and 2). In the broad Naucleeae (Fig. 1: B) all investigated taxa, namely *Cephalanthus*, *Haldina*, *Hallea*, *Sarcocephalus*, *Nauclea*, and *Uncaria*, have capituliform inflorescences and a secondary pollen presentation mechanism.

A few years ago it was shown that the tribe Chiococceae, based on a phylogenetic restriction site analysis (Bremer & Jansen, 1991), is closely related to *Exostema* and *Hintonia*. Later, Bremer (1992) presented morphological data to support a monophyletic unit with Chiococceae, *Exostema* and *Hintonia*, and the subtribe Portlandiinae of the Condamineeae. Within this unit there was support for the monophyly of the Chiococceae s. str., e.g., fleshy fruits with compressed endocarps and one-seeded carpels, but no such support was found for the remaining taxa (subtribe Portlandiinae, *Exostema*, *Hintonia*), and the solution was to include these taxa in a widened Chiococceae. Our data do not contradict a wide Chiococceae, but the inclusion of *Hintonia* is not supported. Delprete has made a more thorough cladistic morphological analysis of the group (presented at the International Conference on the Systematics of the Rubiaceae, 4–6 Oct. 1993, in St. Louis), and he found support for the former subtribe Portlandiinae as the sister taxon to Chiococceae, and suggested it forms a separate tribe. If that is correct, both alternatives (a wide Chiococceae or two separate tribes) are

acceptable and the choice is a matter of taste. An additional result that confirms our earlier findings with morphological data (Bremer & Struwe, 1992) is the inclusion of *Catesbaea*.

The tribe Guettardeae, formerly referred to the subfamily Antirheoideae, is in our analysis related to *Rachicallis* (Hedyotideae) and *Rogiera* (Rondeletieae), and there is no support for a wide circumscription of the subfamily Antirheoideae (Fig. 1). The justification for Robbrecht's circumscription of Antirheoideae seems to be "endosperm soft and oily: embryo frequently very large" (Robbrecht, 1988: 186), but according to our study these characters must have evolved several times in the family. If we force Robbrecht's classification of Antirheoideae (including Vanguerieae, Guettardeae, Chiococceae, Cephalantheae), that grouping requires 50 extra steps (1033 + 50 steps) compared to the most parsimonious trees (1033 steps), and such a solution is thus not very plausible.

The tribe Hamelieae is usually included in the Rubioideae (Verdcourt, 1958; Bremekamp, 1966; Bremer, 1987; Robbrecht, 1988) due to the presence of raphides, a character used to define the subfamily Rubioideae. However, that position was contradicted by an earlier molecular analysis (Bremer & Jansen, 1991) and our data suggest a position within the subfamily Cinchonoideae. The earlier suggestion of Hamelieae as a sister taxon to Argostemmateae (Bremer, 1987) was based on the erroneous assumption that Hamelieae should be part of Rubioideae. Our data support the monophyly of Hamelieae including *Deppea* (Bremer, 1987), also accepted by Robbrecht (1988).

The present analysis also indicates that the neotropical genus *Hillia* is closely related to the Hamelieae and could as well be included in that tribe. It is a genus of often succulent epiphytic shrubs with an interesting taxonomic history. Earlier, *Hillia* was included in the tribe Cinchoneae of the Cinchonoideae (de Candolle, 1830; Schumann, 1891). The seeds of *Hillia* are unique in the family: they have a hair tuft at one end of the seeds, analogous to the hair tuft in many Apocynaceae. *Hillia* also has raphides and right-contorted corolla aestivation, characters that contradict a position in Cinchoneae. Because of these "cardinal characters" Bremekamp moved the genus to Rubioideae as a separate tribe Hillieae (Bremekamp, 1954), and later Verdcourt (1958) accepted the subfamilial position, although he was uncertain about its tribal position. A few years later Bremekamp (1966) even raised it to a new subfamily. In a discussion of testa structures of *Hillia*, in relation to Cinchoneae and Naucleeae, Robbrecht (1988) came to the conclusion that the genus should form a separate tribe within Cinchonoideae. Our results support a position within the Cinchonoideae s. str. clade close to the Hamelieae. This position is in agreement with many morphological characters. All members of the Hamelieae (Bremer, 1987) and *Hillia* have raphides; *Hillia* and the Hamelieae genera *Deppea* and *Omiltemia* also have right-contorted aestivation, which is otherwise rare in the family (Robbrecht, 1988: 83). Bird pollination occurs in *Hillia* subg. *Ravnia* and in *Hamelia*, and succulence is common in *Hillia* and *Hoffmannia*.

THE IXOROIDEAE S.L. (IXOR S.L.) CLADE (Figs. 1 and 2)

The core of this clade is the subfamily Ixoroideae (sensu Robbrecht) but also the tribe Vanguerieae. This close relationship is congruent with the restriction site data (Bremer & Jansen, 1991) but incongruent with an earlier morphological analysis (Bremer & Struwe, 1992). The position of Vanguerieae is controversial, since the morphological data support a relationship to Naucleeae but the molecular data, and also a combination of these data (Bremer, unpublished), strongly support a close relationship to the Ixoroideae s. str. The reason for this incongruency is so far unclear. Within the Ixoroideae s. str. only five genera from three of the five tribes are studied; nevertheless, our data contradict the circumscriptions of the Pavetteae and Gardenieae, respectively. If many more taxa are analyzed, further problems with tribal delimitations appear (Andreasen & Bremer, unpublished). At the base of the Ixoroideae clade we find taxa representing tribes usually included in the Cinchonoideae: *Pogonopus* and *Pinckneya* (Condamineeae), *Calycophyllum* (Cinchoneae), *Pentagonia* (Hippotideae, uncertain subfamily in Robbrecht, 1988), and *Mussaenda* (Isertieae). This association with the Ixoroideae was also shown in earlier phylogenetic analyses based on both restriction site data and morphology (*Pentagonia* was not included) (Bremer & Jansen, 1991; Bremer & Struwe, 1992). It is not possible to say from the present analysis how these taxa are related to each other and to the Ixoroideae; there are several equally parsimonious alternatives from this part of the tree, as indicated by the lack of resolution in the strict consensus tree, and the support for the various branches is low, so many more taxa have to be investigated.

THE RUBIOIDEAE CLADE
(Figs. 1 and 2)

The number of taxa used in this analysis is not representative of the entire subfamily Rubioideae (only seven of sixteen tribes are represented), but the phylogeny corresponds in most parts with the tribes of the Rubioideae (sensu Robbrecht, 1988). However, as previously mentioned, Hamelieae and the genus *Rachicallis* (Hedyotideae) are not parts of the clade, but rather members of the Cinchonoideae s. str. (see above). Robbrecht (1988), in an evaluation of Bremekamp's subfamilies, identified two major groups within Rubioideae: "(i) a number of tribes which have numerous ovules on each placenta (mainly the Hedyotideae, and associated to them: Ophiorrhizeae, Hamelieae, Argostemmateae, Coccocypseleae and Scradereae), and (ii) tribes with solitary erect ovules (numerous tribes, inter alia Psychotrieae and associated tribes) . . . the Anthospermeae, Paederieae, Theligoneae, Spermacoceae and Rubieae." This arrangement of taxa is not congruent with our results. Neither of the two groups is monophyletic according to our data. Within our Rubioideae we can identify two larger clades, one including taxa with few-seeded fleshy fruits (Psychotrieae and Morindeae, but not Anthospermeae, Theligoneae, and Rubieae) and the other group mainly with dry fruits and either numerous (Hedyotideae) or few seeds (Anthospermeae, Rubieae, Theligoneae).

In our analysis (Figs. 1 and 2) *Ophiorrhiza*, a genus with many-seeded capsules, is basal in the tree and is the sister group to all the other Rubioideae. Bremekamp placed *Ophiorrhiza* in a different subfamily (Urophylloideae) because he thought it lacked raphides and also because the testa structures are different (very thick-walled cells vs. thin-walled in Rubioideae). The differences in testa structure can easily be understood if the genus is basal in the subfamily and if the ancestor had the same thick-walled testa cells as in many taxa of the Cinchonoideae.

The next split in our tree goes between the group of taxa with fleshy fruits and few seeds, Psychotrieae and Morindeae, and the remaining taxa. The Psychotrieae and Morindeae branch is supported by a bootstrap fraction of 79% (Fig. 2) and is in agreement with the classification and earlier phylogenetic ideas.

In the large branch including the rest of the subfamily, *Rubia* (Rubieae) and *Theligonum* (Theligoneae) are sister taxa and they together are the sister group to the tribe Hedyotideae. The Hedyotideae form a monophyletic unit, supported by two of the five unique amino acid changes and a bootstrap fraction of 92%. However, in a more extensive analysis of the Rubioideae (Bremer, unpublished) the Hedyotideae become polyphyletic with members of the Spermacoceae nested within the tribe. Within Hedyotideae there are two branches corresponding to the two morphologically identified groups earlier discussed by Bremer (1987). Both of these branches, *Pentas/Parapentas* and *Bouvardia/Oldenlandia/Pentodon*, respectively, are supported by unique amino acid changes and both have 100% bootstrap values. The close affinity between *Rubia* and *Theligonum* is also supported by a unique change of amino acids. *Theligonum* is an aberrant genus with mostly alternate leaves, whereas most Rubiaceae have opposite leaves, and a variable number of stamens (6–30) in the male flowers. Although formerly treated as a separate family, Theligonaceae, it was recently included in the Rubiaceae based on embryological characters (Wunderlich, 1971). In earlier classifications *Theligonum* has been associated with various parts of the angiosperms, e.g., as a separate family within Centrospermae (Eichler, 1878) or Myrtiflorae (Melchior, 1964), or it has been included in Urticaceae (Endlicher, 1836–1841) or Rosaceae (Reichenbach, 1837). Close to the Hedyotideae/Rubieae/Theligoneae in our analysis is the tribe Anthospermeae. This tribe is characterized by unisexual and wind-pollinated flowers; they are unusual characters in the family shared by *Theligonum*. These similarities prompted Wunderlich (1971) to propose a relationship of the Theligoneae to Anthospermeae, but she also pointed to affinities with other tribes of the subfamily Rubioideae, viz. Spermacoceae and Galieae.

For positioning of taxa with uncertain or disputed taxonomic positions, the information provided by *rbc*L sequence data is very useful. In this investigation we included a few genera with uncertain positions. For most of these the positions were the same in all trees and could be positioned with high certainty. *Hillia* (Cinchonoideae) and *Theligonum* (Rubioideae) have already been discussed. Another taxon with disputed tribal position is *Catesbaea* (Catesbaeeae), a West Indian genus of thorny shrubs with single-flowered inflorescences and berries with fleshy to leathery pericarps. Due to its specific kind of fruit it was included in the Gardenieae by Schumann (1891), although Verdcourt (1958) kept it as a separate tribe close to the Gardenieae. Robbrecht & Puff (1986) later removed the tribe from the Gardenieae-related taxa and left it as a "tribus incertae" (Robbrecht, 1988). In our analysis *Catesbaea* becomes the sister taxon to *Cubanola* within the Chiococceae (where it should

be included) clade, a strongly supported branch with a bootstrap value of 96%. This result is congruent with an analysis based on morphology (Bremer & Struwe, 1992), but in conflict with the restriction site analysis, where it was placed on a separate branch close to but outside the Chiococceae s.l. (Bremer & Jansen, 1991).

Another member of Schumann's Gardenieae whose position has been disputed is the large tropical genus *Bertiera*. Verdcourt (1958) was doubtful but kept it close to the Gardenieae (Ixoreae = Pavetteae); Bremekamp (1966) removed it to the subfamily Urophylloideae; and Robbrecht & Puff (1986) excluded it from the Gardenieae-related taxa. The genus is probably not monophyletic (Kirkbride, 1979) but the African species we have investigated in the molecular analysis, *Bertiera breviflora*, is definitely a member of the subfamily Ixoroideae. This relationship is also supported by morphological characters, such as the ixoroid pollen presentation mechanism, left-contorted aestivation, and fleshy fruits.

The genus *Rachicallis* is also of controversial tribal position. It was included in Schumann's (1891) Rondeletieae, but was not mentioned by either Verdcourt (1958) or Bremekamp (1966). However, Robbrecht (1988) listed it under Hedyotideae, perhaps by mistake because in the text it is once mentioned under Rondeletieae (1988: 39). Our results point to a position close to Guettardeae and Rondeletieae. Rondeletieae, however, is not monophyletic and the tribe is in need of revision, as earlier proposed (Bremekamp, 1966).

Pentagonia is a Central and South American genus with large, sometimes pinnatifid-lobed leaves and fleshy fruits with numerous seeds. In Schumann's (1891) classification it was a part of the Mussaendeae (= Isertieae), but Bremekamp (1966) doubted the position and Kirkbride (1979) excluded it from the tribe. In Robbrecht's (1988) classification it was included in the Hippotieae and treated as a "tribus incertae." In our analysis it is placed at the base of the Ixoroideae clade next to the Isertieae (= *Mussaenda*). However, the branches in this part of the tree are weak, with little support, and we should not put much trust in relationships of these branches; perhaps a better sampling or information from another gene would help.

Conclusion

The Rubiaceae are monophyletic and are a sister group to the rest of the Gentianales. With this restricted analysis, representing about eight percent of all genera, we have found that *rbc*L sequencing is very useful for phylogenetic analysis within the family. However, too few taxa have been sequenced to get a complete picture of the family phylogeny. The next goal for our project is to sequence a majority of all genera (so far 165 genera have been sequenced, Bremer & Andreasen, unpublished). Despite the limits in this restricted analysis we can say that the *rbc*L data can be used to test subfamilial and tribal classification in the Rubiaceae and in most parts our results support the present classification based on traditional morphological assessment (Robbrecht, 1988). There is support for the subfamilies Cinchonoideae, Ixoroideae, and Rubioideae, although differently circumscribed. Our data do not support a subfamily Antirheoideae; rather, it is polyphyletic. There is strong support in our data for the following tribes: Naucleeae s.l., Chiococceae s.l., Guettardeae, Hamelieae, Vanguerieae, Hedyotideae, Anthospermeae, and Psychotrieae, but we find no reason to maintain Cephalantheae and subtribe Mitragyninae of the Cinchoneae. We have also found *rbc*L analysis useful in pinpointing genera with uncertain positions, and our results have unequivocally indicated positions for *Bertiera*, *Catesbaea*, *Hillia*, *Ophiorrhiza*, *Rachicallis*, and *Theligonum*.

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CHROMOSOME SURVEY OF THE RUBIACEAE[1]

Michael Kiehn[2]

ABSTRACT

The present knowledge of karyological characters, including basic numbers, ploidy levels, chromosome structures, karyotypes, interphase nuclear types, and DNA contents, and the broad range of variation for most of these characters within Rubiaceae (excluding Rubieae) are summarized. These data are related to the presently existing systematic concepts of the family. The use of karyological characters is exemplified in some taxonomically critical groups: Subfamily Antirheoideae sensu Robbrecht appears heterogeneous with regard to basic numbers and chromosome structure; it contains chromosomally homogeneous tribes (e.g., Vanguerieae or Guettardeae), but also groups with internal variation (such as the tribe Knoxieae or the genus *Chiococca*). Available chromosome data of the tribe Cinchoneae sensu Robbrecht seem to be in accordance with its morphologically and molecular-based subdivision in three tribes: viz. Cinchoneae s. str., Calycophylleae, and Coptosapelteae. The tribe Chiococceae sensu Bremer consists of at least two distinct entities characterized by their chromosome morphology and basic numbers: the former tribe Condamineeae, and the genera *Chiococca* and *Exostema*. A possible subdivision of *Rondeletia* is indicated by three different basic numbers ($x = 9$, $x = 10$, $x = 11$) occurring in the genus, while chromosome data give no hints regarding the position of *Wendlandia* and *Sipanea* in the tribe Rondeletieae. The tribe Isertieae comprises genera with basic numbers of $x = 9$, $x = 10$, and $x = 11$. Thus the tribal position of *Acranthera* ($x = 10$), *Heinsia* ($x = 11$), and *Mycetia* ($x = 11$) cannot be decided on the basis of chromosome numbers alone. *Sabicea* has taxa with either $x = 9$ or $x = 11$; morphological studies are required to check the generic delimitations. The close relationship of *Pauridiantha* and *Urophyllum* is supported by karyomorphology and basic number. A possible origin of the *Spermacoce* out of the tribe Hedyotideae is discussed.

With more than 10,000 species (Mabberley, 1987) the Rubiaceae are one of the larger families in the plant kingdom. Karyological studies in the family show a broad range of variation in chromosome numbers and other chromosomal parameters. Compilations of karyological data, however, are either relatively old (Fagerlind, 1937; Mendes, 1938), regionally restricted (Mangenot & Mangenot, 1958: West Africa; Philip & Mathew, 1988: southern India), or not widely published (Kiehn, 1986a). Relatively numerous counts are published for Africa, India (especially southern India), and New Zealand, and many unpublished data (by the author) exist for Central America and Madagascar. For most other tropical and subtropical areas of the world karyological information on Rubiaceae is scanty. Otherwise, recent chromosome counts of Rubiaceae are scattered in the literature and not easily accessible to taxonomists. Besides the work on the temperate Rubieae, which are not discussed in this survey, only few attempts of more intensive chromosome studies on material of known origin related to larger systematic groups have been made (Hedyotideae: Lewis, 1962, 1965; Anthospermeae: Puff, 1986; *Paederia*: Kiehn, 1991).

Earlier interpretations of chromosome data related to evolutionary trends and classification of Rubiaceae mostly dealt with basic numbers and ploidy levels (e.g., Verdcourt, 1958; Lee & Rink, 1985; Philip & Mathew, 1988). They were based on different systematic groupings of Rubiaceae (Schumann, 1891; Verdcourt, 1958; Wagenitz, 1959; Bremekamp, 1966) because a comprehensive multidisciplinary systematic survey of tropical Rubiaceae has been lacking until recently (Robbrecht, 1988). In the present paper the subfamilial, tribal, and subtribal classification of the Rubiaceae largely follows Robbrecht (1988) but also considers more recent publications that discuss the circumscription and composition of Robbrecht's tribes (e.g., Andersson & Persson, 1991: Cinchoneae, Condamineeae; Bremer, 1992: Chiococceae; Bremer

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& Struwe, 1992: several tribes; Puff & Robbrecht, 1989: Knoxieae; Robbrecht et al., 1991: Morindeae).

The present survey of karyological data in Rubiaceae (excluding Rubieae) is based on a compilation of all data available from the literature until 1993 and on unpublished data of the author. It is hoped that this study will stimulate a more intensive use of karyological data for phylogenetic and systematic considerations in the family.

Materials and Methods

Original chromosome data reported in this paper are based on field fixations (*) or fixations from plants cultivated at the Botanical Garden of the University of Vienna, Austria (H.B.V.), or at the National Tropical Botanical Garden in Kauai, Hawaii, U.S.A. (NTBG) (**): *Chiococca alba* (L.) Hitchcock**: origin unknown, seeds from Major Howell Botanical Garden (1984), cult. in H.B.V. sub RR-814 (WU). *Chiococca bermudiana* S. Brown**: Bermuda Island. Spitall Pond, 1978, *Phillips s.n.* (K, WU). *Chiococca coriacea* Mart. & Gal.**: Mexico. Veracruz: Balzopote, 22 Mar. 1986, *D. Lorence 4972*, cult. in NTBG sub 880087 (PTBG, WU). *Pauridiantha paucinervis* (Hiern) Bremek. subsp. *lyalii* (Baker) Verdc.**: Madagascar. Prov. Diégo Suarez: Massif du Marojezy, NW of Mantantenina village, 24 July 1985, *Puff et al. 850724-1/3* (WU). *Sipanea hispida* Benth. ex Wernh.**: Brazil. Distrito Federal: Córrego Bananal (15°35'S, 47°55'W), *Kirkbride 5229* (US). *Thysanospermum diffusum* Champ.**: China. Jiangxi: Mt. Jiulian, seeds from Botanical Garden Long Wu Lu Shanghai (1983-539) (WU, Herb. Botanical Garden Long Wu Lu Shanghai). *Urophyllum ellipticum* Thw.*: Sri Lanka. About 5 km N of Gilimale on the road to Carney, S slope of Adam's Peak, 10 Feb. 1984, *Lorenz EL-100284-18/1* (K, WU). *Urophyllum zeylanicum* Thw.**: Sri Lanka. Above Hakgala Sanctuary Botanical Gardens (about 6 km SE of Nuwara Eliya), 12 Feb. 1984, *Lorenz EL-120284-25/4* (K, WU).

Materials and methods for original data of DNA measurements are listed in Kiehn (1986a), with additional explanation in Kiehn (1991).

Suitable tissues and methods for obtaining reliable chromosome data for Rubiaceae (counts, chromosome morphology, DNA contents) are described in Kiehn (1986a, 1991). Due to the presence of tannins in many Rubiaceae, self-tanning effects are often encountered in chromosome fixations, resulting in prefixation and/or clumping of chromosomes, or influencing staining conditions (see Greilhuber, 1988, for the discussion of these problems). Thus, Giemsa staining (of Guerra, 1983) or Feulgen staining (after hydrolysis in 5 N HCl for 50 min. at 20°C) is not possible in many cases. Often additional staining with aceto-carmine (2% in 45% acetic acid) at room temperature is required, especially when dealing with meiotic divisions.

Difficulties in obtaining proper karyological results due to small size and/or relatively high numbers of chromosomes can be (at least partly) avoided by a pretreatment of actively growing meristematic tissues with 8-hydroxy-chinolin (4–6 hr., 8–10°C) before fixation.

The basis for the discussion of chromosome numbers is a critical list of chromosome counts for Rubiaceae (excluding Rubieae) from original literature (Kiehn, 1986a), including more than 300 counts by the author. The checklist is continually updated, and will be published separately. It comprises about 2000 records for nearly 1000 taxa. It is sorted according to Robbrecht's classification and contains critical remarks on taxonomic and nomenclatural problems, and the reliability of deviating counts. The author welcomes suggestions and additions (e.g., unpublished counts, which can be added to the list). Excerpts of the actual list are available on request.

Survey of Actual Knowledge

Chromosome Numbers

Due to determination problems and inconsistent nomenclature (both leading to the misnaming of investigated taxa), and also the difficulties in obtaining proper karyological results (many taxa having small chromosomes that clump together, tannins often interfering with fixation and/or staining procedures, etc.), older chromosome data for Rubiaceae must be looked at with caution. A critical checklist of chromosome counts in Rubiaceae (excluding Rubieae) contains chromosome data for about 10% of all Rubiaceae species (Kiehn, 1986a, unpublished). There are counts for at least one taxon of all tribes and subtribes of Robbrecht's classification except for Triainolepideae, Lathraeocarpeae, Aulacocalyceae, and Jackieae (Table 1).

Chromosome numbers in Rubiaceae (excluding Rubieae) range from $2n = 12$ (e.g., in *Houstonia*) to $2n = 220$ (or even higher) in *Coprosma* and *Lasianthus*, basic numbers from $x = 6$ to $x = 17$ (Table 1); a report of $x = 29$ for *Hedyotis scandens* Roxb. (Sarkar et al., 1982) has to be confirmed. The predominant basic number is $x = 11$ (many woody Rubioideae, Ixoroideae, Antirheoideae, Cin-

Table 1. Chromosome basic numbers and ploidy levels in the tribes and subtribes of Robbrecht's (1988) classification of Rubiaceae (except Rubieae) in subfamilial arrangement. Doubtful literature data are in parentheses.

	Investigated genera/taxa	Basic numbers	Ploidy levels
1. CINCHONOIDEAE			
Cinchoneae			
Cinchoninae	9/22	11, 13, 14, 17, (18)	2x, 4x, 6x
Mitragyninae	2/7	11	4x
Naucleeae			
Adininae	4/5	11	4x
Neolamarckiinae	1/1	11	4x
Naucleinae	2/5	11	4x, 8x
Hillieae	1/1	17-18	2x
Henriquezieae	3/3	7?, 8?	4x?
Rondeletieae	6/17	9, 10, 11, (12, 14)	2x, 4x
Sipaneeae	1/2	11	2x, 4x
Condamineeae	2/3	?11, 12	2x
Isertieae	8/47	9, 10, 11	2x, 4x, ?6x
Urophylleae	1/2	9	6x
Pauridiantheae	1/3	9	2x
2. IXOROIDEAE			
Gardenieae			
Diplosporinae	2/5	11	2x
Gardeniinae	27/64	(10), 11, 17	2x, 4x, 6x
Pavetteae	7/82	(10), 11	2x, 3x, 4x, 6x
Coffeeae	2/65	11	2x, 3x, 4x, 6x, 8x
Aulacocalyceae	—		
Hypobathreae	7/8	11, (12)	2x, 4x
3. ANTIRHEOIDEAE			
Retiniphylleae	1/2	?10	2x
Vanguerieae	12/35	11	(2x), 4x
Guettardeae	5/9	11	4x, 6x, 8x
Chiococceae	1/4	(11), 12, 13, 14	2x (4x)
Alberteae	2/2	?10, 11	4x
Cephalantheae	1/1	11	4x
Craterispermeae	1/1	11	2x
Knoxieae	4/12	10, (11, 12)	2x, 4x
4. RUBIOIDEAE			
Hedyotideae	24/167	6, 7, 8, 9, 10, 11, 13, 15, 17	2x, 4x, 6x, 8x, 12x
Ophiorrhizeae	1/10	11, (12)	2x, 4x
Coccocypseleae	1/6	10	2x, 4x
Argostemmateae	1/3	11, 14	2x
Hamelieae	3/14	12, 14	2x, 4x, 6x, 8x, 12x
Schradereae	2/2	11	2x
Psychotrieae	12/127	(10), 11	2x, 4x, 6x, 8x, 10x, 12x
Triainolepideae	—		
Lathraeocarpeae	—		
Morindeae	4/16	(5), 11	2x, 4x, 8x, 20-22x
Coussareae	2/2	?11	2x
Paederieae	9/34	(10), 11, (12, 13)	2x, 4x, 6x, 8x
Anthospermeae			
Anthosperminae	5/62	11	2x, 4x, 6x, 8x
Coprosminae	3/79	(10), 11	(2x), 4x, 6x, 8x, 12x, 14x, 20x
Operculariinae	2/2	11	2x
Theligoneae	1/2	10, 11	2x
Spermacoceae	12/51	8, (10, 11), 14, 15, 17	2x, 4x, 6x

Table 1. Continued.

	Investigated genera/taxa	Basic numbers	Ploidy levels
Tribus incertae			
Catesbaeae	1/2	12	2x
Hippotieae	2/2	10?	4x
Tammsieae	1/1	?	4x
Jackieae	—		
Perameae	1/1	9	2x, 8x

chonoideae pro parte). Without other morphological or karyological information this number gives no hints for systematic groupings or relationships in Rubiaceae. Other basic numbers, however (from $x = 6$: *Houstonia*, up to $x = 17$: Cinchonoideae pro parte), may be useful for phylogenetic considerations (see discussion below).

Ploidy levels in Rubiaceae range from $2x$ to 20–$22x$ (Table 1). While some taxonomic groups seem to be characterized by a certain ploidy level (e.g., Vanguerieae; see discussion below), polyploid series are often encountered in herbaceous groups like the Hedyotideae, but also occur in predominantly woody tribes such as the Psychotrieae. They sometimes seem to have originated in parallel fashion even within genera (e.g., *Anthospermum*: Puff, 1986; *Paederia*: Kiehn, 1991; *Psychotria*: Kiehn, 1986a).

Polyploidization of single cells ($2x \rightarrow 4x$, or $4x \rightarrow 8x$) in seedling root tips has been observed several times (Kiehn, 1986a: 189, and literature cited there). Mitotic divisions of such cells are regular and lead to series of cells with the higher ploidy level, which also have larger cell volumes (and thus have chromosomes that are easier to count). Reports from such cells may be the reason for single $4x$ reports concerning taxa normally reported to be diploid.

OTHER KARYOLOGICAL PARAMETERS

There are several notable features regarding Rubiaceae chromosome structures:

Normally, length and structure of all chromosomes within a diploid set are more or less uniform (Fig. 1: *Sipanea hispida* Benth. ex Wernh.); usually there are one or two pairs of satellite chromosomes. However, chromosome length and structure can differ considerably among rubiaceous taxa. Chromosomes can be characteristic for a given group or even for a taxon: members of the Vanguerieae, for example, have small compact chromosomes hardly exceeding a length of 0.5 μm in metaphase, while chromosomes of Pavetteae taxa might measure as much as 3 μm in mitotic metaphase and are well structured longitudinally.

There are some remarkable exceptions to the uniformity of chromosome sets normally found in Rubiaceae; the implications of heteromorphic karyotypes in *Urophyllum* and *Pauridiantha* (Fig. 2) are discussed below.

Structural changes of the chromosomes during the cell cycle can be characteristic for a certain group; for example, Spermacoceae have late condensation of comparatively large terminal (telomeric) parts of the chromosomes, a condensation type unusual in Rubiaceae, and can be distinguished from other Rubioideae by this character.

Karyotypes of Rubiaceae have been analyzed in some publications on the basis of conventionally stained material (e.g., Bhattacharyya, 1958: *Gardenia*; Sharma & Chatterjee, 1960: *Ixora*; both dealing with ornamental taxa). In these papers, secondary constrictions and structural characters of the chromosomes have been described and used to discuss relationships between the corresponding taxa. But the investigated chromosomes were not fully contracted (cf. descriptions and drawings of chromosomes). Thus these "structures" depend on the state of chromosome contraction and not primarily on their structural constitution, with the result that these studies cannot be reproduced.

Modern banding techniques have only been used in a few instances. Giemsa banding has been successfully applied to a few members of the Gardenieae (*Gardenia* sp., Kiehn, unpublished; *Genipa americana* L., Guerra, 1993, Kiehn, unpublished) while in other cases (Psychotrieae, Anthospermeae) these techniques failed, partly because of small chromosome size, partly due to the interference of tannins present in these groups (Kiehn, 1986a). Thus, the use of Giemsa sequential staining or other banding techniques for systematic purpose in Rubiaceae seems to be limited.

Data on interphase nuclear types of members of the family are very scanty (de Poucques, 1949;

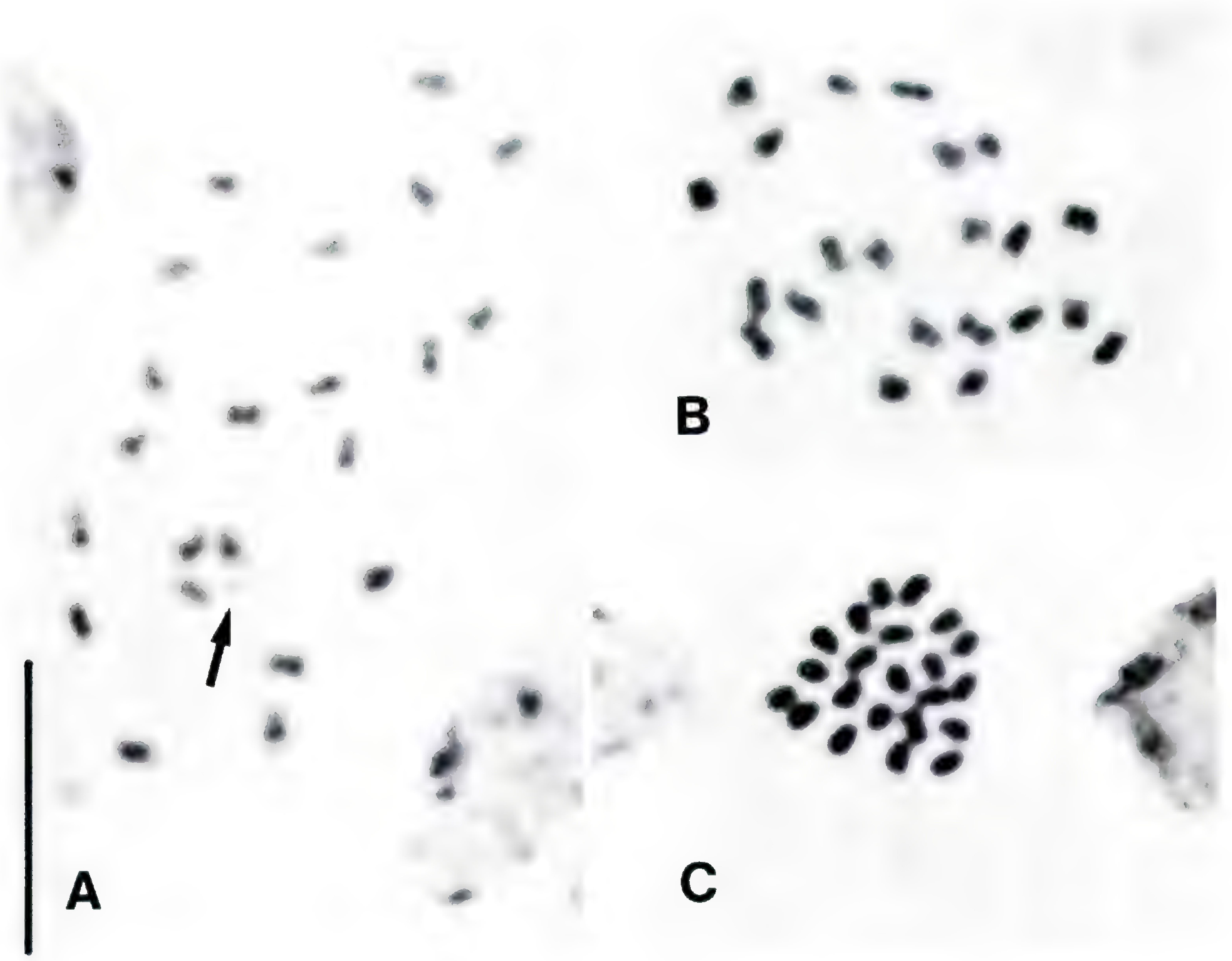


Figure 1. Mitotic chromosomes of *Sipanea hispida (Kirkbride 5229)*: $2n = 22$.—A. Prophase.—B. Prometaphase.—C. Metaphase. Arrow indicates satellite. Bar = 10 μm.

Kiehn, 1986a), but are useful to characterize certain groups (e.g., Spermacoceae: Kiehn, 1986a).

DNA amounts for 49 species of Rubiaceae have been established (Kiehn, 1986a; Bennett & Smith, 1991; Cros et al., 1994). As would be expected from the small chromosome size of most Rubiaceae, these amounts are in the lower third of the range of angiosperm nuclear DNA values (cf. Bennett & Smith, 1976, 1991; Bennett et al., 1982). Between the lowest and highest 2C DNA amount of Rubiaceae there is a 9.6-fold range (Table 2). While whole groups (e.g., Vanguerieae) seem to be quite uniform in their DNA amounts, DNA values can differ remarkably between taxa of the same basic number and ploidy level, suggesting both DNA increase (e.g., *Hamelia, Pavetta*) and DNA decrease (e.g., Vanguerieae, *Nauclea*).

Implications of Chromosome Data

ANTIRHEOIDEAE SENSU ROBBRECHT (1988)

Table 3 summarizes the available chromosomal data known for tribes attributed to subfamily Antirheoideae. The only two counts for neotropical

Table 2. Lowest and highest known DNA values within Rubiaceae; cf. Kiehn (1986a). pg = picogram.

Total 2C-value	lowest	1.05 pg	*Serissa foetida*
	highest	10.13 pg	*Anthospermum spathulatum* subsp. *spathulatum*
1C-value per haploid genome (x)	lowest	0.1513 pg	*Vangueriopsis lanciflora*
	highest	0.7575 pg	*Hamelia patens*
Mean DNA-value per chromosome	lowest	0.0138 pg	*Vangueriopsis lanciflora*
	highest	0.0631 pg	*Hamelia patens*

Figure 2. Chromosomes of :—A. *Pauridiantha paucinervis* subsp. *lyalii* (*Puff et al. 850724–1/3*) : $2n = 18$.—B. *Urophyllum ellipticum* (*Lorenz EL-100284–18/1*) : $n = 27$.—C. *Urophyllum zeylanicum* (*Lorenz EL-120284–25/4*) : $2n = 54$. Bar = 10 μm.

Retiniphylleae suggest $x = 10$ on $2x$ as ploidy level. On the other hand, Vanguerieae are comparatively well known cytologically. With one exception, all data indicate $x = 11$ on a $4x$ level. Therefore, the deviating taxon (*Cuviera acutiflora* DC.) should be reinvestigated. The Vanguerieae are uniform regarding other chromosome characters, such as chromosome length and structure, interphase nucleus structure, and DNA amount.

Taxa from five genera of Guettardeae have been investigated karyologically: $x = 11$ is the general basic number; tetraploidy is found in four genera. Only the Hawaiian genus *Bobea* is represented by taxa on the hexa- and octoploid level. Chromosome structure and length differ from the Vanguerieae, mitotic metaphase chromosomes being more elongated and measuring nearly 1 μm.

The taxonomic concept for the Chiococceae is under discussion. *Exostema* has been removed from the Cinchoneae and placed in the Condamineeae by Andersson & Persson (1991). This view is supported by Bremer (1992), who included the Condamineeae and *Exostema* in the tribe Chiococceae. Chromosomal data for Chiococceae sensu Bremer (1992) are summarized in Table 4. Unpublished counts by the author reveal $2n = 28$ for *Chiococca bermudiana* and $2n = 26$ for *C. coriacea*, while an exact number for *C. alba* could not be established ($2n$ between 24 and 28). Literature data suggest $x = 12$ on $2x$, and $x = 11$ on $4x$ level for this taxon. Chromosomes of *Chiococca* have prominent secondary constrictions and show some tendency to break at the centromeres. The chromosomes of *Exostema* are similar in structure to

Table 3. Chromosome basic numbers and ploidy levels in the tribes and genera attributed to the Antirheoideae sensu Robbrecht (1988). Doubtful literature data are in parentheses.

Genus [numbers of investigated taxa]	Basic number	Ploidy level
Retiniphylleae	?10	2x
Retiniphyllum [2]	?10	2x
Vanguerieae	11	(2x), 4x
Canthium [10]	11	4x
Cuviera [2]	11	(2x), 4x
Keetia [4]	11	4x
Lagynias [1]	11	4x
Pachystigma [1]	11	4x
Peponidium [1]	11	4x
Plectronia [2]	11	4x
Psydrax [2]	11	4x
Pygmaeothamnus [1]	?	min. 4x
Rytigynia [1]	11	4x
Vangueria [9]	11	4x
Vangueriopsis [1]	11	4x
Guettardeae	11	4x, 6x, 8x
Antirhea [1]	11	4x
Bobea [3]	11	6x, 8x
Dichilanthe [1]	11	4x
Guettarda [3]	11	4x
Timonius [1]	11	4x
Chiococceae	(11), 12, 13, 14	2x, (4x)
Chiococca [4]	(11), 12, 13, 14	2x, (4x)
Alberteae	?10, 11	4x
Alberta [1]	11	4x
Nematostylis [1]	10–11	4x
Cephalantheae	11	4x
Cephalanthus [2]	11	4x
Craterispermeae	11	2x
Craterispermum [1]	11	2x
Knoxieae	10, (11, 12)	2x, 4x
Chorochorion [1]	10	4x
Knoxia [2]	10, (11, 12)	2x, 4x
Paraknoxia [1]	10	2x
Pentanisia [8]	10	2x, 4x

the ones of *Chiococca*, and its reported basic numbers are $x = 11$ (one count by Fagerlind, 1937, on material cultivated at Kew), $x = 13$ (Kiehn, unpublished; McDowell, unpublished), and $x = 14$ (McDowell, unpublished). *Portlandia* and *Cubanola* are characterized by large chromosomes and most probably have $x = 12$ as the basic number. Thus, the tribe Chiococceae sensu Bremer (1992) is heterogeneous from the chromosomal point of view, and karyology provides distinctive characters: While *Portlandia* and *Cubanola* are doubtlessly closely related regarding their chromosome morphology, *Chiococca* chromosomes are different in structure. A possible relationship between *Chiococca* and *Exostema*, strengthened by the similar appearance of chromosomes and common unusual basic numbers, should be investigated further.

Results for Alberteae and Cephalantheae indicate tetraploidy on $x = 11$. The interphase nuclear type of *Cephalanthus* differs from the one characteristic for Vanguerieae and resembles the type of Rondeletieae, Sipaneeae, or Naucleeae.

The situation for Knoxieae has become more diverse since the survey of Puff & Robbrecht (1989). Besides diploid and tetraploid representatives on $x = 10$, there are also reports with $x = 11$ and $x = 12$. No data on chromosome morphology or interphase nuclei are available. More studies are needed to clarify the karyological situation in this tribe and its systematic implications.

In conclusion, the Antirheoideae sensu Robbrecht (1988) are heterogeneous as regards their karyological characters.

CINCHONEAE S.L.

In Table 5 the actual knowledge of chromosome data in genera placed in the Cinchoneae s.l., excluding those transferred to the Hedyotideae such as *Bouvardia*, is listed. Recently, Andersson & Persson (1991) emended Cinchoneae and Coptosapelteae and proposed a new tribe, Calycophyl-

Table 4. Chromosome numbers and ploidy levels of the Chiococceae sensu Bremer (1992). Doubtful data are in parentheses.

Genus [number of investigated species]	Basic number/ ploidy level	Position sensu Robbrecht (1988)	Position sensu Andersson & Persson (1991)	Position sensu Bremer (1992)
Chiococca [4]	(11), 12, 13, 14/2x (4x)	Chiococceae	—	Chiococceae
Cubanola [1]	12/2x	Condamineeae	—	Chiococceae
Exostema [4]	11, 13, 14/2x	Cinchoneae–Cinchoninae	Condamineeae?	Chiococceae
Portlandia [2]	?11, 12/2x	Condamineeae	—	Chiococceae

Table 5. Chromosome numbers and ploidy levels of the Cinchoneae s.l. Doubtful data are in parentheses.

Genus [number of investigated species]	Basic number; ploidy level	Position sensu Robbrecht (1988)	Position sensu Andersson & Persson (1991)	Position sensu Bremer (1992)
Calycophyllum [1]	17; 2x	Cinchoneae-Cinchoninae (Cin-C)	Calycophylleae	—
Cinchona [5]	17; 2x (4x)	Cin-C	Cinchoneae (Cin)	—
Coptosapelta (incl. *Thysanospermum*) [1]	10; 2x	Cin-C	Coptosapelteae (Cop)	—
Cosmibuena [1]	17–18; 2x	Cin-C	Cin	—
Crossopteryx [1]	11; 2x	Cin-C	Cop	—
Exostema [4]	11, 13, 14; 2x	Cin-C	Condamineeae?	Chiococceae
Hillia [1]	17–18; 2x	Hillieae	Hillieae	—
Hymenodictyon [3]	11; 2x; 4x; 6x	Cin-C	Cop	—
Ladenbergia [2]	17(–18); 2x	Cin-C	Cin	—
Luculia [3]	11; 4x	Cin-C	Cop	—
Mitragyna (incl. *Hallea*) [2]	11; 4x	Cinchoneae-Mitragyninae (Cin-M)	Cop	—
Remijia [1]	17; 6x	Cin-C	Cin	—
Uncaria [5]	11; 4x	Cin-M	Cop	—

leae, based on a cladistic analysis of morphological characters. A basic number of $x = 17$ is limited to their first clade including Cinchoneae and Calycophylleae (Table 6; the other genera of this clade have not yet been investigated karyologically). The division between the three tribes of this clade and the other taxa is supported by additional karyological data, such as structural differences between chromosomes, e.g., of *Cinchona* and *Hymenodictyon* (Kiehn, 1986a; Ehrendorfer, 1988: fig. 12).

The taxa in the emended tribe Coptosapelteae all have $x = 11$ with ploidy levels ranging from 2x to 6x (Table 5), except one count for *Thysanospermum* (= *Coptosapelta*) *diffusum* Champ. with $x = 10$. This taxon probably does not belong to this clade (Robbrecht, pers. comm.).

RONDELETIEAE-SIPANEEAE (TABLE 7)

Within *Rondeletia* s.l., *Rondeletia* s. str. and its satellites *Rogiera* and *Arachnothryx* possibly are separated from each other by different basic numbers (Kiehn, 1986a). Whether $x = 9$ really is characteristic for *Arachnothryx*, $x = 10$ for *Rogiera*, and $x = 11$ for the remaining taxa of *Rondeletia* should be verified by additional studies. As the report of $x = 14$ and diploidy for a cultivated *Rondeletia* (Sarkar et al., 1982) is doubtful, all taxa of this complex apparently are tetraploids.

All investigated taxa of *Simira* and *Warscewiczia* are also tetraploids; their chromosomes are similiar in structure. The strange number of $2n = 42$ in *Simira* (Kiehn, 1986a) most probably is the result of a fusion of two pairs of chromosomes of an original set of $2n = 44$.

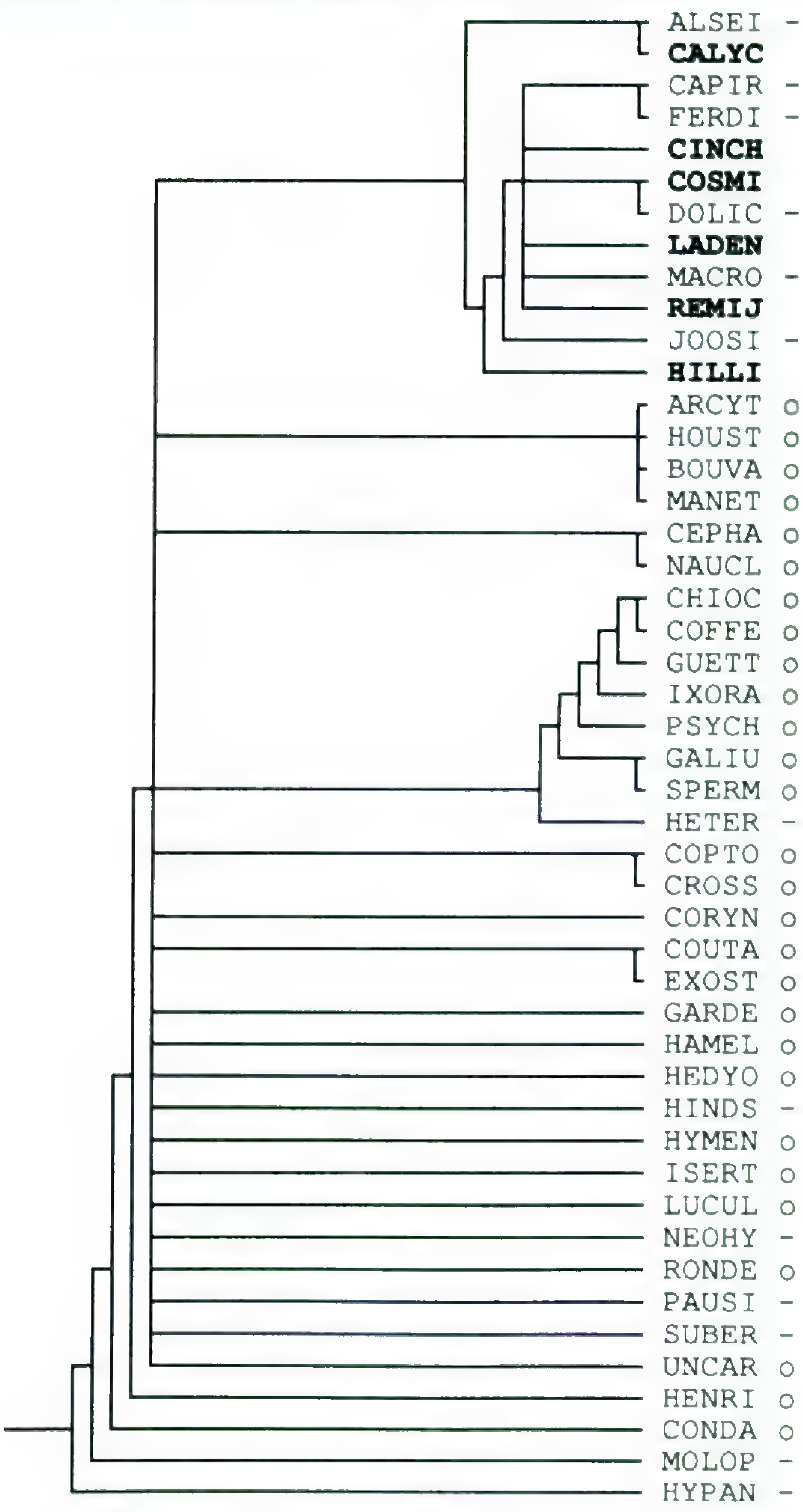
Wendlandia is the only genus of the Rondeletieae (in this circumscription) with diploid taxa: it is also the only paleotropical one. Chromosomes of *Wendlandia* are generally smaller than those of the remaining Rondeletieae. These karyological data, however, are no help for the discussion of the placement of *Wendlandia* in Rondeletieae.

In *Sipanea*, one diploid and one tetraploid taxon on a basic number of $x = 11$ are known. While chromosomes of mitotic metaphase (Fig. 1C) are not elongated as in Rondeletieae but rather compact as in Naucleeae, interphase nuclear structures of *Sipanea* resemble those of both Rondeletieae and Naucleeae.

ISERTIEAE (TABLE 8)

Different basic numbers occur in the Isertieae. The investigated genera with exclusively neotropical distribution, *Gonzalagunia* and *Isertia*, have $x = 9$ and $x = 10$, respectively (Kiehn, 1986a, 1987), and are tetraploids. *Sabicea* occurs both in the Neo- and the Paleotropics. All its investigated taxa are tetraploids. In the Neotropics only species of *Sabicea* with $x = 11$ have been found so far, while in the Paleotropics species with both $x = 11$ and $x = 9$ exist. Within a genus this is very unusual for non-herbaceous Rubiaceae and might indicate two different entities. Thus detailed morphological investigations are required to clarify the generic delimitation of *Sabicea*. The investigated taxa of

Table 6. Groups with x = 17 in the strict consensus tree with hypothetical outgroup from the cladistic analysis of morphological characters by Andersson & Persson (1991). "—" not investigated, "O" other basic numbers.



the genera *Mussaenda* and *Pseudomussaenda* are diploids on a basic number of $x = 11$; one literature record of $2n =$ ca. 70 for *Pseudomussaenda* obviously is wrong. The tribal position of *Mycetia* ($x = 11$, $2x$ and $4x$), *Heinsia* ($x = 11$, $2x$), and *Acranthera* ($x = 10$, $2x$), currently included in Isertieae, cannot be determined on the basis of chromosome numbers. *Acranthera*, however, has prometaphase chromosomes of ca. 3 μm (Kiehn, 1986b: fig. 1c), which is unusually large for Isertieae.

PAURIDIANTHEAE–UROPHYLLEAE

Urophyllum and *Pauridiantha* share a karyological character not reported for any other Rubiaceae: they have a heteromorphic set of chromosomes. That means that both long and short chromosomes are present in the karyotype (Fig. 2). Together with their common basic number of $x = 9$, this might support the idea of a close relationship between the two genera as suggested by morphological studies (Buchner, 1995).

HEDYOTIDEAE–SPERMACOCEAE

Recent chloroplast DNA studies (Bremer et al., 1995; Natali et al., 1995) provide strong evidence for the Spermacoceae having evolved out of the Hedyotideae, thus making the Hedyotideae paraphyletic. Karyological data could support this assumption: Hedyotideae reveal a broad diversity of chromosome basic numbers and ploidy levels, while Spermacoceae predominantly have a basic number of $x = 14$ and are diploids or tetraploids (Kiehn, 1986a). All recent models for the origin of $x = 14$ in Spermacoceae interpret this as a paleotetraploid state from basic numbers existing in Hedyotideae by loss or rearrangements of chromosomes: on a basis of $x = 7$ by Majumdar (1975), who observed secondary associations of bivalents in

Table 7. Chromosome basic numbers and ploidy levels in the genera of the Rondeletieae and Sipaneeae of Robbrecht's (1988) classification of Rubiaceae. Doubtful literature data are in parentheses.

Genus [number of investigated species]	Basic number	Ploidy level
RONDELETIEAE	9, 10, 11, (12, 14)	2x, 4x
Arachnothryx [2]	9?	4x
Rogiera [2]	10?; 11?	4x
Rondeletia [2]	11; 14?	(?2x), 4x
Simira [2]	2n = 42 derived from x = 11	4x
Warszewiscia [1]	10–11	4x
Wendlandia [8]	11; 12?	2x
SIPANEEAE	11	2x, 4x
Sipanea [2]	11	2x, 4x

Table 8. Chromosome basic numbers and ploidy levels in the genera attributed to the Isertieae in Robbrecht's (1988) classification of Rubiaceae. Doubtful literature data are in parentheses.

Genus [number of investigated species]	Basic number	Ploidy level
Acranthera [1]	10	2x
Gonzalagunia [3]	9	4x
Heinsia [1]	11	2x
Isertia [3]	10	4x
Mussaenda [27]	11	2x
Mycetia [2]	11	2x, 4x
Pseudomussaenda [2]	11	2x, (6x)
Sabicea [8]	9; 11	4x

Spermacoce (however, no Hedyotideae with x = 7 that are morphologically similar to Spermacoceae are known); or on a basis of $x = 9$ by Kiehn (1986a), who considered the unusual numbers x = 17 (in the African genus *Otiophora*, recently included in Spermacoceae by Igersheim & Rohrhofer, 1993), $x = 16$ and $x = 15$ (occasional in Spermacoceae) as possible intermediates.

The recently published basic number of $x = 8$ on the diploid level in the neotropical genus *Galianthe* (Daviña & Cabral, 1991) offers another possibility for a paleotetraploid origin of $x = 14$: from $2n = 4x = 32$ down to $2n = 30$ (reported for the *Galianthe*-related genus *Triodon*, which has been sunk into *Galianthe* by Cabral & Bacigalupo, 1993) to $2n = 28$. This theory, however, would implicate a different origin for the $x = 17$ of *Otiophora*, thus making the Spermacoceae polyphyletic.

Conclusions

In combination with the results of other research fields (e.g., molecular biology, morphology, and phytogeography), karyological characters have proven to be useful for understanding the relationships in taxonomically difficult groups of Rubiaceae. Moreover, they can help to explain evolutionary lines within the family. Therefore, published karyological data for Rubiaceae must be made more easily accessible for taxonomists and systematists, the exchange of information between different fields of research should be improved, and multidisciplinary research efforts on larger taxonomic groups should include chromosome studies.

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TRIBES AND GENERA OF THE CINCHONEAE COMPLEX (RUBIACEAE)[1]

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Abstract

The phylogenetic relationships of taxa recently referred to the tribes Calycophylleae, Cinchoneae, and Hillieae were analyzed using cladistic methods. The analyses used morphological and micromorphological characters, and a selection of 60 species were used as terminal taxa. It was concluded that all three tribes are supported as monophyletic but must be altered somewhat in circumscription. The tribes Cinchoneae and Hillieae are sister groups, and these two in turn form the sister group of the Calycophylleae. The tribe Calycophylleae should comprise the genera *Alseis, Calycophyllum, Capirona, Ferdinandusa, Macrocnemum, Wittmackanthus,* and the new genus *Semaphyllanthe* L. Andersson here segregated from *Calycophyllum.* These genera are all supported as monophyletic, although the support is sometimes weak. The tribe Hillieae should comprise *Balmea, Blepharidium, Cosmibuena,* and *Hillia.* It was not tested whether or not these genera are monophyletic. The tribe Cinchoneae should comprise *Cinchona, Joosia, Ladenbergia, Maguireocharis, Pimentelia, Remijia, Stilpnophyllum,* and the new genus *Cinchonopsis* L. Andersson here erected for *Cinchona amazonica. Dolicholobium* occurs as a basal clade of the Cinchoneae but its position is unstable and it is possibly not related to this alliance at all. *Cinchona* is supported as monophyletic if *C. amazonica* and *C. henleana* are excluded, the latter one being transferred to *Ladenbergia. Ladenbergia* is supported as monophyletic in approximately the traditional sense. *Remijia* is supported as monophyletic if *Cephalodendron* is included. *Joosia* and *Stilpnophyllum* are supported as monophyletic, while the remaining genera are monotypic. Six new combinations are made: *Semaphyllanthe intonsa* (Steyerm.) L. Andersson, *S. megistocaula* (K. Krause) L. Andersson, *S. merumensis* (Steyerm.) L. Andersson, *S. obovata* (Ducke) L. Andersson, *S. spectabilis* (Steyerm.) L. Andersson, *S. venezuelensis* (Steyerm.) L. Andersson, and *Cinchonopsis amazonica* (Standley) L. Andersson.

The tribe Cinchoneae is often widely circumscribed (see, e.g., de Candolle, 1830; Schumann, 1891, 1897; Robbrecht, 1988). In this sense it is a pantropical group comprising all genera with multiovulate ovaries, ascendingly imbricate ovules, and winged seeds. The case was examined by Andersson & Persson (1991), who concluded that a number of genera traditionally included in the Cinchoneae are not at all closely related to *Cinchona.* Out of 50 genera referred to the Cinchoneae by Robbrecht (1988), 10 were transferred to the Hedyotideae and six to the Condamineae. Disregarding four genera left as incertae sedis, the remainder were divided among four tribes. The paleotropical genera, except *Dolicholobium,* were referred to the tribe Coptosapelteae. Four genera, *Alseis, Calycophyllum, Schizocalyx,* and *Wittmackanthus,* were referred to the new tribe Calycophylleae. *Hillia* was left in a monotypic tribe of its own, the Hillieae. Thirteen genera were left in the Cinchoneae: *Capirona, Cephalodendron, Cinchona, Cosmibuena, Dolicholobium, Ferdinandusa, Joosia, Ladenbergia, Macrocnemum, Maguireocharis, Pimentelia, Remijia,* and *Stilpnophyllum.* The relationships between these four tribes were not resolved in strict consensus although a majority of equally parsimonious solutions suggested that the Coptosapelteae are possibly the sister group of the three neotropical tribes, which in turn may form a monophyletic group. The tribes Cinchoneae and Hillieae are probably sister groups, forming together the sister group of the Calycophylleae.

The investigation of Andersson and Persson was based in large part on data from the literature, using genera as terminal taxa. The aim of the present study is to reach higher resolution regarding circumscription and interrelationships of the neotropical tribes and genera of the Cinchoneae complex, using original observations throughout and species as terminal taxa.

Two initial assumptions are based on the findings of Andersson & Persson: (1) the neotropical tribes Calycophylleae, Cinchoneae, and Hillieae form a monophyletic group, possibly including also the genera *Balmea* and *Blepharidium;* (2) the sister group of this complex is the tribe Coptosapelteae. Neither of these assumptions was fully supported,

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but they are accepted here in lack of a better hypothesis.

The genus *Schizocalyx* was included in the Calycophylleae by Andersson & Persson but, the fruit and seed characters now having been examined, it appears that its closest relative is *Bathysa* in the Rondeletieae. It is therefore excluded from this study.

Materials and Methods

Observations of morphological features were made on herbarium material, occasionally supplemented by pickled material. Species descriptions were used as a basis for coding and were based on numerous collections; a list of representative material studied is given in Appendix 1. Most vegetative characters were observed on dry material, supplemented by label data. Vegetative buds, flowers, and seeds were boiled in water before dissection and observation.

Leaf blade anatomy was observed in material cut by hand from boiled leaves and examined under the interference contrast microscope without staining.

Observations on the sculpturing of the inside of exotesta cells were made with the aid of the scanning electron microscope (SEM), using material treated in the following way. Rehydrated seeds were stored in 70% ethanol and transferred to tap water before treatment. The outer exotesta wall was oxidized by treatment in 15% HNO_3 on a boiling water bath for 5 min. The seeds were then rinsed twice in distilled water and treated with a sonicator at 8 microns. No treatment time was found that suited all materials, and a series of treatments varying between 1 and 20 seconds was therefore tried for each sample. After sonication the seeds were rinsed in water, transferred briefly to 96% ethanol, and left to dry on a slide. Generally, only one seed sample from each species was studied.

Pollen characters were studied with the aid of the SEM, using the methods described by Andersson (1993). Generally, only one sample per species was studied.

The cladistic analyses were performed using the PAUP software package (Swofford, 1993). Numerous preliminary runs were made to guide the selection of taxa and characters as well as the coding of characters. The final runs, on which the following discussion is based, were divided into two steps.

Step I was designed to reveal major subdivisions in the complex, i.e., mainly aiming at establishing tribal delimitations. For this a matrix was used comprising a small number of representatives (Fig. 1) from all "generic level" groups found in the preliminary runs. In this first series *Hymenodictyon floribundum* was used as outgroup, being a taxon mostly included in the Coptosapelteae in the investigation by Andersson & Persson (1991), and one for which a complete data set was available. Four characters were deleted in Step I: 2, 43, 47, and 57.

Step II was designed to achieve better resolution and a more detailed hypothesis concerning the "*Cinchona* clade," clade 60 of Figure 1. For this purpose a matrix was used containing ca. 10 species each of the larger genera and all species of the smaller genera for which sufficient data were available (Fig. 2). Two species found in Step I to belong to basal clades of the sister group were used as a joint outgroup, *Ferdinandusa elliptica* and *Macrocnemum roseum*. Thirteen characters were deleted in Step II: 13, 14, 16, 29, 31, 32, 33, 35, 39, 46, 56, 59, and 60.

The 60 terminal taxa were selected arbitrarily from ca. 120 species actually investigated in order to minimize the amount of missing data.

The selection of characters was based on experience from an ongoing species level revision, and all recorded characters found to vary among species and being shared by at least two species were included in preliminary runs. Characters found in preliminary runs to be highly homoplasious were then excluded. All characters and states are listed in Table 1.

PAUP was run with the following optional heuristic search settings: addition sequence = simple, 1 tree held at each step, TBR branch swapping performed, MULPARS option in effect, steepest descent option not in effect, MAXTREES = 1000, multistate taxa interpreted as polymorphism. Quantitative characters (4, 11, 27, 30, 48, 58) were treated as ordered, others as unordered. After a first run with unweighted characters, the characters were reweighted by the maximum value of the rescaled consistency index. Successive reweighting was then performed until reaching a constant number of trees and identical consensus trees in two successive runs. In step two, monophyly of the outgroup was secured by reinforcing a monophyly constraint tree.

A complete character matrix is given in Table 2; also included in this table is a list of acronyms with corresponding species names and authorities. The results of the cladistic analyses were analyzed with the aid of the MacClade package (Maddison

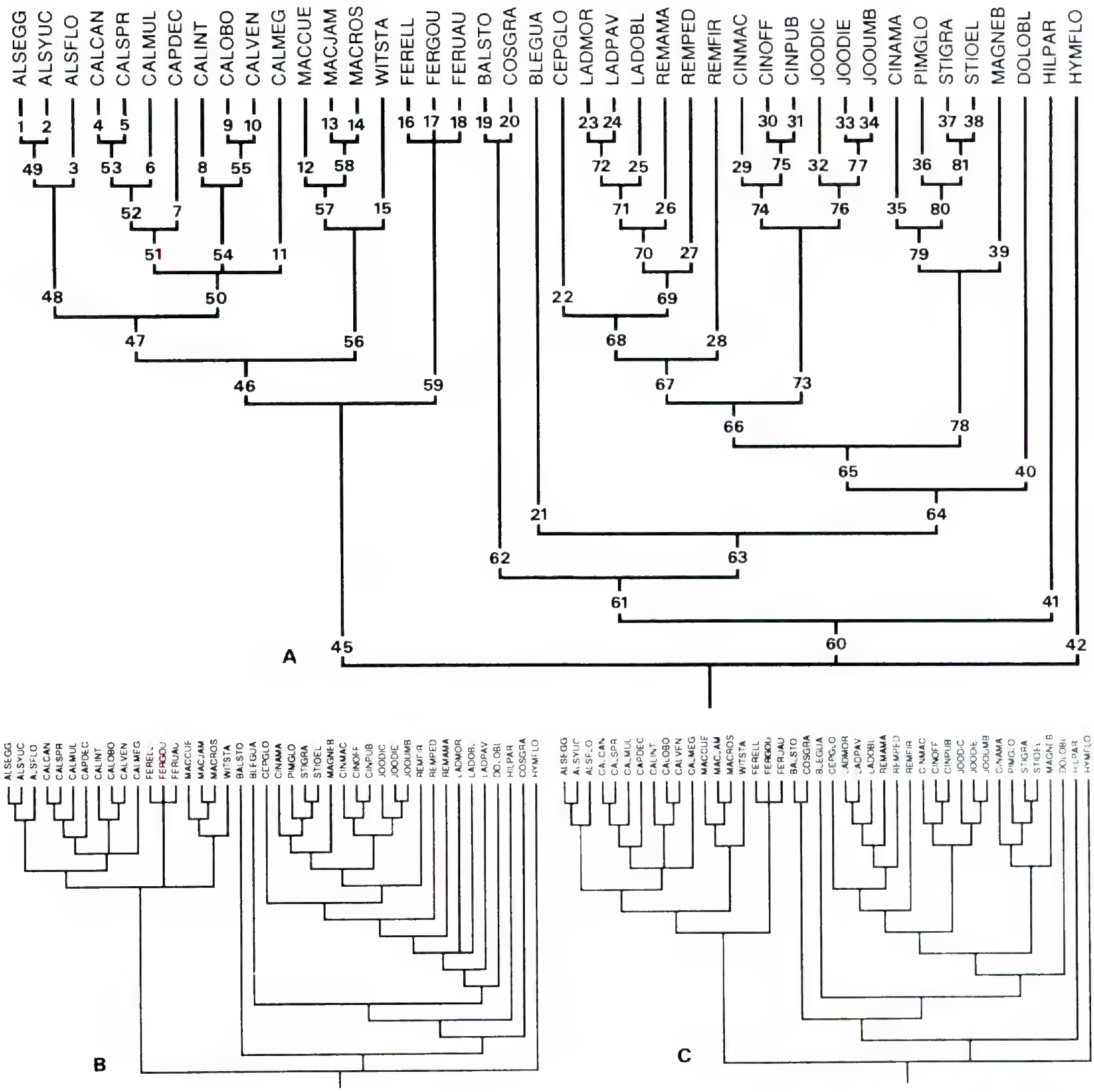

Figure 1. Trees from the Step I analyses.—A. One out of nine equally parsimonious minimal trees resulting after successive reweighting. Figures on edges are ordinal numbers used in the discussion and the apomorphy list.—B. Strict consensus of the 24 minimal trees resulting from the search based on equally weighted characters.—C. Strict consensus of the 9 minimal trees resulting after successive reweighting. Zero length edges are collapsed. Acronyms are explained in the legend of Table 2. A complete list of apomorphies of tree A is provided in Appendix 2.

& Maddison, 1992), which was also used for numbering of edges of the trees presented here.

The Characters

The characters and character states are listed in Table 1. For the most part this is assumed to be self-explanatory, and comments on morphology and codings will be given here only where such are supposed to be needed.

Class limits for quantitative characters were set in order to minimize the number of multistate taxa.

Data on growth habit are based entirely on label data and these are with few exceptions rather superficial. It appears, however, that while the species of some genera are typically very tall trees at flowering, growing often to a height of 30 or 40 m, those of others are shrubs or small to medium-sized trees. A critical limit seems to be whether or not a species may attain a height of over 20 m. The difference between a shrubby and a treelike growth form, on the other hand, seems less taxon specific. Character 1, therefore, expresses growth

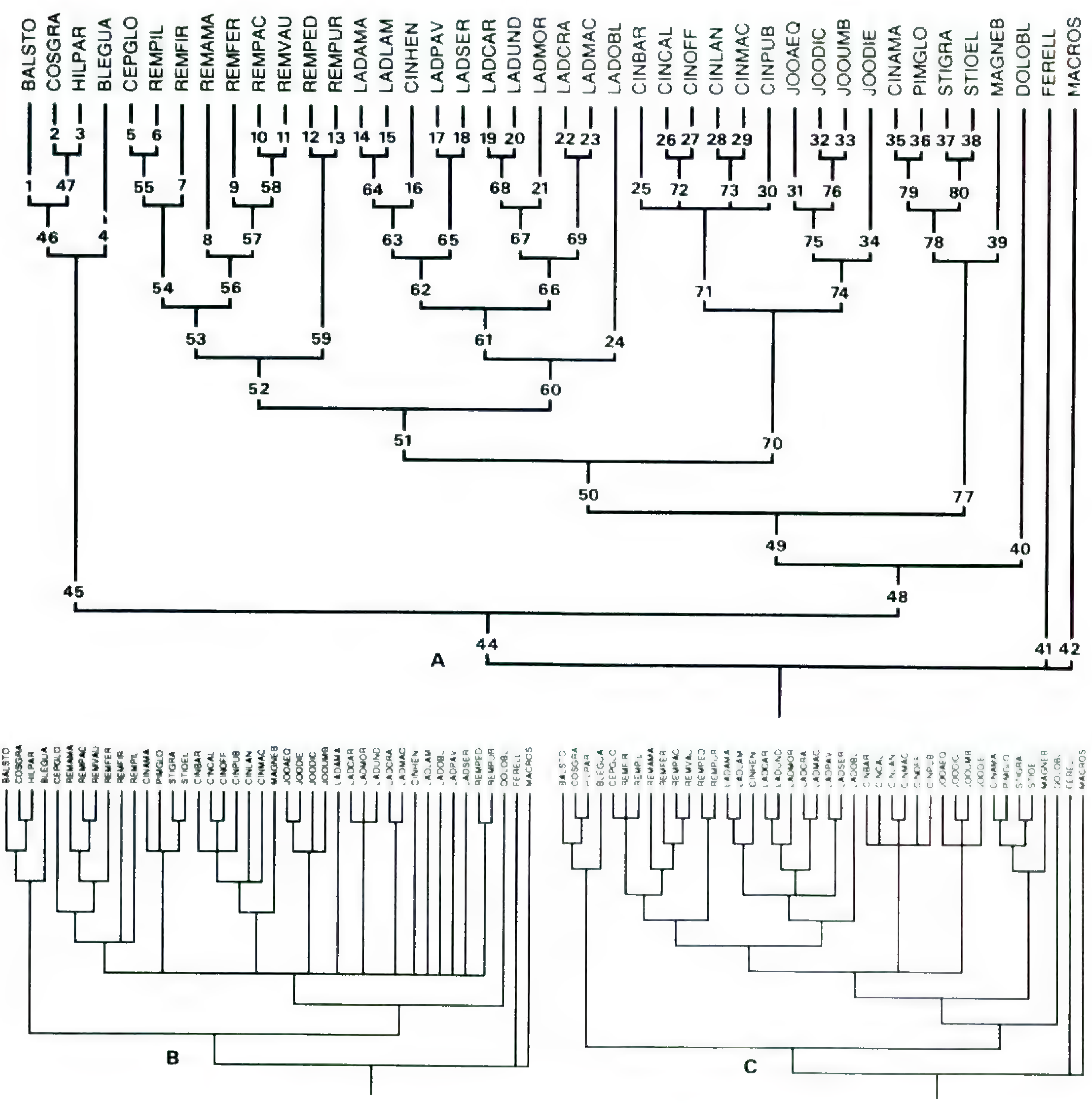

Figure 2. Trees from the Step II analyses.—A. One out of 569 equally parsimonious minimal trees resulting after successive reweighting. Figures on edges are ordinal numbers used in the discussion and the apomorphy list.—B. Strict consensus of 1000 minimal trees saved from the search based on equally weighted characters.—C. Strict consensus of the 569 minimal trees resulting after successive reweighting. Zero length edges are collapsed. Acronyms are explained in the legend of Table 2. A complete list of apomorphies of tree A is provided in Appendix 2.

capacity rather than actual size. The figure used is the maximum height recorded for a species.

Although it may be suspected that an epiphytic or chasmophytic growth habit is typical of the tribe Hillieae (as delimited below), label data are generally too poor to make this character useful.

Stipules are regarded as caducous if shed by the formation of an abscission layer. By this definition, caducous stipules may occasionally be shed rather late and then persist for most of the life span of the leaf. On the other hand, stipules of *Joosia* are persistent in the sense used here, although they soon die off and become much eroded. Typically, persistent stipules remain attached to the branch long after the leaf is shed.

Stipules are initiated as lateral leaf base appendages. They soon widen circumnodally, either in interpetiolar or in intrapetiolar direction, or both. Interpetiolar and intrapetiolar fusion thus seem to depend on independent growth processes and are therefore coded as independent characters.

Two different types of stipules occur with respect

Table 1. Characters and character states.

Vegetative morphology

- 1—growth habit
  - 1 tall trees (to >20 m)
  - 2 smaller trees, or shrubs
- 2—phyllotaxy
  - 1 decussate
  - 2 whorled
- 3—stipule persistence
  - 1 caducous
  - 2 persistent
- 4—stipules: intrapetiolar fusion
  - 1 free
  - 2 partly fused
  - 3 fused throughout
- 5—stipule posture in bud
  - 1 held flat back-to-back
  - 2 clasping to contorted
  - 3 overlapping
- 6—domatia
  - 1 absent
  - 2 tuft or pouch (-with-tuft) domatia present
  - 3 pit or crypt domatia present
- 7—density of secondary leaf veins
  - 1 <15 pairs per 5 cm of midrib
  - 2 ≥15 pairs per 5 cm of midrib

Inflorescence morphology

- 8—terminal inflorescence
  - 1 present
  - 2 absent
- 9—partial inflorescences ("principal inflorescence branches")
  - 1 lax, dichasial or mainly so
  - 2 congested into headlike clusters, dichasial or mainly so
  - 3 ± purely monochasial
  - 4 thyrsoid
  - 5 raceme- or spike-like
  - 6 of solitary flowers
- 10—bracts and bracteoles
  - 1 present, plane and ± triangular
  - 2 present, ± sacciform
  - 3 absent

Floral morphology

- 11—flower size
  - 1 small, corolla tube + lobe <10 mm long
  - 2 medium, corolla tube + lobe 10–25 mm long
  - 3 large, corolla tube + lobe 25–55 mm long
  - 4 very large, corolla tube + lobe >55 mm long
- 12—heterostyly
  - 1 absent
  - 2 present
- 13—protogyny
  - 1 absent
  - 2 present
- 14—calyx shape
  - 1 tubular at base, with lobes 0.5–2× the length of tube
  - 2 tubular at base, with lobes <0.5× the length of tube (or lobes sometimes obsolete)
  - 3 tubular at base, with lobes >2× the length of tube
  - 4 ± closed in young bud, opening by a slit ("spathaceous")
  - 5 of free lobes
  - 6 reduced to minute teeth or totally obsolete
- 15—colleter placement inside calyx
  - 1 in a row at base
  - 2 solitary or in small groups at sinuses
  - 3 in groups or solitary toward base opposite sinuses
  - 4 scattered ± all over
  - 5 numerous on lobe margins at base
  - – coded as missing when colleters are lacking
- 16—calycine semaphylls
  - 1 absent
  - 2 present at least in some flowers of an inflorescence
- 17—corolla aestivation
  - 1 valvate
  - 2 contorted
  - 3 imbricate
  - 4 open
  - 5 reduplicate
- 18—corolla shape
  - 1 campanulate
  - 2 salverform
  - 3 urceolate
  - 4 funnel-shaped
- 19—corolla color
  - 1 white to cream, sometimes pale greenish
  - 2 pink to reddish or purplish
- 20—corolla indumentum outside
  - 1 absent or sparse
  - 2 sericeous
  - 3 ± densely hirtellous
- 21—corolla tube indumentum inside
  - 1 absent
  - 2 hirsute above stamen attachments, with long, rigid hairs
  - 3 ± hirtellous or villosulous to villose above stamen attachments
  - 4 ± densely pubescent below stamen attachments
- 22—corolla tube surface inside
  - 1 smooth
  - 2 papillose above stamen attachments or throughout
  - 3 papillose only below stamen attachments
  - 4 coarsely verruculose
- 23—linear thinnings in corolla tissue between sinus base and filament attachment
  - 1 absent
  - 2 present
- 24—corolla lobe indumentum adaxially
  - 1 glabrous
  - 2 shortly papillose-hirtellous along margins

Table 1. Continued.

3 villous along margins, with long, thin-walled hairs
4 ± densely villous or hirsute all over, with thick-walled hairs

25—terminal, wedge-shaped part of corolla lobes
1 absent or inconspicuous
2 conspicuous

26—marginal, petaloid appendages of corolla lobes
1 absent
2 present

27—filament attachment
1 to basal ¼ of corolla tube
2 around middle (¼–¾ of corolla tube)
3 to distal ¼ of corolla tube

28—filament length
1 long (± equaling anther or longer)
2 short
3 long in brevistylous, short in macrostylous flowers

29—filament indumentum
1 absent
2 filaments hirsute to villous

30—anther attachment
1 dorsifixed around middle
2 dorsifixed near base
3 basifixed

31—anther presentation
1 anthers included to semi-exserted
2 anthers wholly exserted

32—anther shape
1 elongate to linear (length/width ratio ≥2)
2 button-shaped (length/width ratio <2)

33—anther base
1 obtuse
2 sagittate

34—apical connective process
1 absent
2 present

35—dorsally widened and reinforced connective
1 absent
2 present

36—disc indumentum
1 absent
2 present

37—style indumentum
1 absent
2 hirsute or puberulous distally or throughout
3 hirsute or puberulous at the very base, otherwise glabrous

38—stigma branches
1 straight, long (on the average ≥15% of style length)
2 straight, short (on the average <15% of style length)
3 strongly recurved
4 absent, stigma ± capitate

39—placenta
1 attached centrally by a lamella
2 attached apically by a stipe

Fruit morphology

40—septicidal dehiscence
1 present, basipetal
2 present, acropetal
3 absent

41—loculicidal dehiscence
1 absent
2 present, partial
3 present, ± complete

42—apical, beaklike process in mature fruit
1 absent
2 present

43—endocarp
1 thin (<0.4 mm thick)
2 thick

44—calyx presence
1 calyx present (but often much eroded) in fruit
2 calyx abscised in fruit

Seed morphology

45—hilum position
1 central or subcentral on seed body
2 marginal
3 on wing

46—seed wing shape
1 wing completely (but sometimes partly narrowly) surrounding seed body
2 wing interrupted at seed body
3 rudimentary and irregularly developed

47—seed wing margin
1 entire, irregularly lobed, or ± dented
2 deeply laciniate
3 densely ciliate/fimbriate

48—seed length (including wing)
1 small (< 4.0 mm)
2 medium (4.0–11 mm)
3 long (> 11 mm)

Vegetative anatomy

49—raphides
1 absent
2 present

50—adaxial leaf epidermis
1 1-layered
2 2- to several-layered

51—adaxial leaf cuticle
1 thin
2 thick (approaching half of epidermal cell depth, or thicker)

Pollen

52—aperture type
1 colporate with distinct colpus
2 colporate with indistinct colpus
3 porate

Table 1. Continued.

53—colpus ends
  1 acute
  2 obtuse to truncate
54—os
  1 clearly demarcated with regular, often slightly thickened margin
  2 poorly demarcated with irregular margin
55—tectum perforation type
  1 reticulate (lumen/muri ratio >1.0)
  2 foveolate (lumen/muri ratio <1.0)
  3 imperforate
56—lumen differentiation into two distinct size classes
  1 absent
  2 present
57—latitudinal differentiation of lumina
  1 absent, or lumina smaller toward colpi
  2 lumina larger toward colpi
58—columella length
  1 long (≥ 0.5 μm)
  2 medium (0.15–0.4 μm)
  3 short (< 0.15 μm)

Seed anatomy

59—wall thickenings of inner tangential wall
  1 meshwork or broad bands
  2 plate with large pores
  3 walls not thickened, or thickening an undifferentiated sheet
60—spinules or verruculae on secondary wall inside
  1 absent
  2 present

to their posture in bud. They are either held flat back-to-back, and are then usually lingulate to obovate and herbaceous in texture, or they are clasping to contorted, forming a cone over vegetative buds, and then more or less triangular and usually brownish and more or less coriaceous in texture. When the stipules are fused throughout both inter- and intrapetiolarly to form a calyptra, this character does not apply and was coded as missing data.

In *Joosia* the branching of partial inflorescences is predominantly monochasial, more or less boragoid shoot chains arising as lateral branches from a basal dichasial branching. In *Joosia aequatoria* and *J. dielsiana*, with more or less congested inflorescences, the boragoid branches are often short and few-flowered. In these cases it is dubious whether inflorescence branching should be coded as dichasial or monochasial, occasional monochasial branchings being common in cymes of the Rubiaceae. Based on observations of a number of collections of *J. dielsiana* with longer inflorescence branches it was concluded that this ambiguous situation has arisen through reduction of longer monochasial branch systems. The inflorescence branches of *J. aequatoria* and *J. dielsiana* are therefore coded as monochasial.

In *Remijia* inflorescences are typically thyrsoid. In several species, like *R. pilosinervula*, where the thyrses are few-tiered, reduction series occur, from several-tiered to 1-tiered thyrses. In the extreme such inflorescences may appear to be highly condensed dichasia. In such cases these inflorescences were coded as thyrsiform, however.

When the available flowering collections are few it may be impossible to decide whether the species is heterostylous. Macrostylous flowers in heterostylous species are not strikingly different from flowers of monomorphic species, and in some of the heterostylous species examined the number of macrostylous plants may exceed that of brevistylous ones by one to ten. The following coding practice was followed. In genera where heterostyly was documented to be common (*Cinchona*, *Joosia*, *Ladenbergia*, and *Remijia*), the species were coded as heterostylous if the brevistylous morph was encountered, otherwise as missing data. In other genera, where heterostyly has never been documented, species were coded as homostylous on the basis of single observations of long-styled flowers.

In *Dolicholobium* the flowers are functionally unisexual (on monoecious plants), with the nonfunctional organs vestigial. Because this character state is autapomorphic in the present data set, it was not included in the analysis and *D. oblongifolium* was coded as homostylous, the difference in style development between flower morphs being obviously non-homologous with heterostyly in other genera.

The presence of colleters on the inside of the calyx is highly unpredictable, even within species. When present, however, the number and placement of colleters is fairly constant within species. Therefore, states were assigned to placement and grouping of colleters, while absence of colleters was coded as missing data.

Corolla aestivation in some species of *Cosmibuena* was described as quinquncial by Taylor

Table 2. Complete data matrix. Acronyms: ALSEGG = *Alseis eggersii* Standley, ALSFLO = *A. floribunda* Schott in Sprengel, ALSYUC = *A. yucatanensis* Standley, BALSTO = *Balmea stormae* Martínez, BLEGUA = *Blepharidium guatemalense* Standley, CALCAN = *Calycophyllum candidissimum* (M. Vahl) DC., CALINT = *C. intonsum* Steyerm., CALMEG = *Calycophyllum megistocaulum* (Krause) C. M. Taylor, CALMUL = *C. multiflorum* Griseb., CALOBO = *C. obovatum* (Ducke) Ducke, CALSPR = *C. spruceanum* (Benth.) J. D. Hook., CALVEN = *C. venezuelense* Steyerm., CAPDEC = *Capirona decorticans* Spruce, CEPGLO = *Cephalodendron globosum* Steyerm., CINAMA = *Cinchona amazonica* Standley, CINBAR = *C. barbacoensis* Karsten, CINCAL = *C. calisaya* Wedd., CINLAN = *C. lancifolia* Mutis, CINHEN = *C. henleana* Karsten, CINMAC = *C. macrocalyx* Pavón ex DC., CINOFF = *C. officinalis* L., CINPUB = *C. pubescens* M. Vahl, COSGRA = *Cosmibuena grandiflora* (Ruiz López & Pavón) Rusby, DOLOBL = *Dolicholobium oblongifolium* A. Gray, FERELL = *Ferdinandusa elliptica* Pohl, FERGOU = *F. goudotiana* Schumann, FERUAU = *F. uaupensis* Spruce ex Schumann, HILPAR = *Hillia parasitica* Jacq., HYMFLO = *Hymenodictyon floribundum* (Hochst. & Steud.) Robinson, JOOAEQ = *Joosia aequatoria* Steyerm., JOODIC = *J. dichotoma* (Ruiz López & Pavón) Karsten, JOODIE = *J. dielsiana* Standley, JOOUMB = *J. umbellifera* Karst., LADAMA = *Ladenbergia amazonensis* Ducke, LADCAR = *L. carua* (Wedd.) Standley, LADCRA = *L. crassifolia* (DC.) Standley, LALDLAM = *L. lambertiana* (C. Martius) Klotzsch in Hayne, LADMAC = *L. macrocarpa* (M. Vahl) Klotzsch in Hayne, LADMOR = *L. moritziana* Klotzsch in Hayne, LADOBL = *L. oblongifolia* (Mutis) L. Andersson, LADPAV = *L. pavonii* (Lambert) Standley, LADSER = *L. sericophylla* Standley, LADUND = *L. undata* Klotzsch in Hayne, LUCINT = *Luculia intermedia* Hutch., MACCUE = *Macrocnemum cuencanum* Standley, MACJAM = *M. jamaicense* L., MACROS = *M. roseum* (Ruiz López & Pavón) Wedd., MAGNEB = *Maguireocharis neblinae* Steyerm., PIMGLO = *Pimentelia glomerata* Wedd., REMAMA = *Remijia amazonica* Schumann, REMFER = *R. ferruginea* (A. St. Hil.) DC., REMFIR = *R. firmula* (C. Martius) Wedd., REMPAC = *R. pacimonica* Standley, REMPED = *R. pedunculata* (Karsten) Flückiger, REMPIL = *R. pilosinervula* Steyerm., REMPUR = *R. purdieana* Wedd., REMVAU = *R. vaupesiana* Steyerm., STIGRA = *Stilpnophyllum grandifolium* L. Andersson, STIOEL = *S. oellgaardii* L. Andersson, WITSTA = *Wittmackanthus stanleyanus* (R. M. Schomb.) Kuntze.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ALSEGG	2	1	1	1	2	1	1	12	5	1	1	1	2	3	?	1	4	3	1	1
ALSFLO	2	1	1	1	2	1	1	1	5	1	1	1	2	3	?	1	4	3	1	1
ALSYUC	1	1	1	1	2	1	1	1	5	1	1	1	2	3	2	1	4	3	1	1
BALSTO	2	1	1	1	1	1	1	1	1	1	3	1	1	3	?	1	2	2	2	1
BLEGUA	1	1	1	1	2	12	1	2	1	1	34	1	1	1	2	1	3	2	1	1
CALCAN	1	1	1	3	?	3	1	1	1	2	1	1	1	6	?	2	3	1	1	1
CALINT	1	1	1	1	2	1	1	1	1	1	1	1	1	1	3	2	2	1	2	1
CALMEG	1	1	1	1	2	12	1	1	1	1	1	1	1	2	?	1	2	1	1	1
CALMUL	1	1	1	3	?	2	1	1	1	2	1	1	1	6	?	1	3	1	1	1
CALOBO	1	1	1	1	2	1	1	1	1	1	1	1	1	1	?	2	2	1	1	1
CALSPR	1	1	1	3	?	3	1	1	1	2	12	1	1	6	?	1	3	1	1	1
CALVEN	2	1	1	1	2	1	1	1	1	1	1	1	1	1	2	2	2	1	1	1
CAPDEC	1	1	2	3	?	2	1	1	1	1	3	1	1	2	4	2	2	1	12	1
CEPGLO	2	1	1	12	1	1	1	2	2	1	23	2	1	2	?	1	1	2	12	2
CINAMA	2	1	1	2	1	2	1	1	1	1	1	1	1	3	?	1	1	1	1	2
CINBAR	2	1	1	1	1	1	1	12	1	1	12	2	1	2	3	1	1	2	2	2
CINCAL	2	1	1	12	1	3	1	1	1	1	12	2	1	1	?	1	1	2	2	2
CINLAN	2	1	1	1	1	12	1	1	1	1	2	2	1	2	3	1	1	2	2	2
CINHEN	2	1	1	2	1	1	1	1	1	1	3	2	1	2	4	1	1	2	1	2
CINMAC	2	1	1	1	1	2	1	1	1	1	2	2	1	13	3	1	1	2	2	2
CINOFF	2	1	1	1	1	3	1	1	1	1	2	2	1	1	?	1	1	2	2	2
CINPUB	2	1	1	1	1	1	1	1	1	1	2	2	1	12	?	1	1	2	2	2
COSGRA	2	1	1	2	1	1	1	1	1	1	4	1	1	2	3	1	2	2	1	1
DOLOBL	2	1	1	2	1	1	1	2	1	3	34	1	1	2	?	1	2	2	1	2
FERELL	1	1	1	1	2	1	1	1	1	1	3	1	1	3	1	1	2	2	1	1
FERGOU	2	1	1	1	2	1	1	1	1	1	23	1	1	3	1	1	2	2	1	1
FERUAU	2	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	2	2	1	1
HILPAR	2	1	1	1	1	1	1	1	6	1	4	1	1	5	?	1	2	2	1	1
HYMFLO	2	1	1	1	2	1	1	1	5	1	1	1	1	3	?	1	1	3	2	1
JOOAEQ	2	1	2	1	1	1	1	1	3	3	3	2	1	1	?	1	1	2	2	2
JOODIC	2	1	2	1	1	1	1	1	3	3	2	2	1	4	?	1	1	2	1	2

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
ALSEGG	2	1	1	1	1	1	1	1	2	1	2	2	1	1	1	1	2	3	2	1
ALSFLO	2	1	1	1	1	1	1	1	2	1	2	2	1	1	1	1	2	3	2	1
ALSYUC	2	1	1	1	1	1	1	1	2	1	2	2	1	1	1	1	2	3	2	1
BALSTO	1	1	1	1	1	1	3	2	1	2	1	1	2	1	1	1	1	2	1	1
BLEGUA	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	1	1	1	1	1
CALCAN	2	1	1	1	1	1	3	1	1	1	2	2	1	1	1	2	1	3	1	1
CALINT	2	1	1	1	1	1	1	1	2	1	2	2	1	2	1	1	1	1	1	1
CALMEG	2	1	1	1	1	1	2	1	2	1	2	2	1	1	1	1	1	1	1	1
CALMUL	2	1	1	1	1	1	3	1	1	1	2	2	1	1	1	1	1	1	1	1
CALOBO	2	1	1	1	1	1	2	1	2	1	2	2	1	1	1	1	1	1	1	1
CALSPR	2	1	1	1	1	1	3	1	1	1	2	2	1	1	1	1	1	3	1	1
CALVEN	2	1	1	1	1	1	2	1	2	1	2	2	1	1	1	1	1	1	1	1
CAPDEC	2	1	1	1	1	1	12	1	2	3	1	1	1	1	1	1	1	1	1	1
CEPGLO	4	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
CINAMA	3	1	1	4	1	1	2	2	1	2	1	1	1	1	1	2	1	1	1	2
CINBAR	1	1	2	3	1	1	12	3	1	2	1	1	1	1	1	1	1	1	1	2
CINCAL	1	1	2	3	1	1	2	3	1	2	1	1	1	1	1	2	1	1	1	2
CINLAN	1	1	2	3	1	1	2	3	1	2	1	1	1	1	1	1	1	1	1	2
CINHEN	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	2
CINMAC	13	1	2	3	1	1	2	3	1	2	1	1	1	1	1	1	1	1	1	2
CINOFF	1	1	2	3	1	1	2	3	1	2	1	1	1	1	1	1	1	1	1	2
CINPUB	13	1	2	3	1	1	2	3	1	2	1	1	1	1	1	1	1	1	1	2
COSGRA	1	1	1	1	1	1	3	2	1	23	1	1	2	1	1	1	12	2	1	1
DOLOBL	1	1	1	1	1	1	2	2	1	3	1	1	1	1	1	1	1	1	1	1
FERELL	1	1	1	1	1	1	2	1	1	1	2	2	1	1	2	1	1	2	1	1
FERGOU	1	1	1	1	1	1	2	1	1	1	2	2	1	1	2	1	1	2	1	1
FERUAU	1	1	1	1	1	1	2	1	1	1	2	2	1	1	2	1	1	2	1	1
HILPAR	1	1	1	1	1	1	3	2	1	3	1	1	1	1	1	1	1	2	1	1
HYMFLO	1	1	1	1	1	1	2	2	1	2	1	1	2	2	1	1	1	4	1	3
JOOAEQ	1	1	1	1	1	2	2	2	1	2	1	1	1	1	1	2	1	1	1	1
JOODIC	1	1	1	1	1	2	2	2	1	2	1	1	1	1	1	2	1	1	1	1

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
ALSEGG	2	1	1	1	3	2	1	2	1	1	1	1	1	1	1	1	1	2	1	2
ALSFLO	2	1	1	1	3	2	1	2	1	1	1	1	1	1	1	1	1	1	1	2
ALSYUC	2	1	1	1	3	2	1	2	1	1	1	1	1	1	1	1	1	2	1	2
BALSTO	2	2	1	2	1	1	1	12	2	1	1	1	2	1	2	1	1	2	1	1
BLEGUA	2	2	1	2	1	1	1	2	1	2	1	3	?	1	3	1	1	?	1	1
CALCAN	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	2	1	1
CALINT	2	1	1	1	1	1	1	2	1	2	1	1	1	1	1	1	1	1	1	2
CALMEG	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2
CALMUL	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2
CALOBO	2	1	1	1	1	1	2	2	1	1	2	1	1	1	1	1	1	1	1	2
CALSPR	2	1	1	1	1	2	1	2	1	1	1	1	1	1	1	1	1	2	1	2
CALVEN	2	1	1	1	1	1	2	2	1	1	2	1	1	1	1	1	1	1	1	2
CAPDEC	1	1	1	1	3	1	1	2	1	2	1	13	1	1	1	1	1	1	1	2
CEPGLO	2	1	1	2	1	1	1	2	1	1	12	1	2	2	2	1	1	2	1	1
CINAMA	1	1	1	1	1	1	1	2	1	2	1	1	2	2	2	1	1	2	1	1
CINBAR	1	1	1	1	1	1	1	3	1	2	1	?	?	?	?	1	?	?	?	?
CINCAL	1	1	1	1	1	1	1	2	1	12	1	1	2	2	2	1	1	2	1	1
CINLAN	1	1	1	1	1	1	1	2	1	1	2	?	?	?	?	1	?	?	1	1
CINHEN	1	1	1	1	1	1	1	23	1	1	1	?	?	?	?	1	?	2	1	1
CINMAC	1	1	2	1	1	1	1	2	1	1	2	1	2	2	2	1	1	2	1	1
CINOFF	1	1	?	1	1	1	1	2	1	2	1	1	2	2	2	1	1	2	?	?
CINPUB	1	1	1	1	1	1	1	2	1	2	1	1	2	2	2	1	1	2	1	1
COSGRA	1	2	1	2	3	1	1	2	2	2	1	2	2	1	23	1	1	3	1	1
DOLOBL	1	1	1	1	?	1	1	2	1	2	1	3	?	1	2	1	1	3	1	1
FERELL	2	1	12	1	1	1	1	3	1	1	2	1	1	12	1	2	1	1	1	1
FERGOU	1	1	1	1	1	12	1	3	1	1	1	1	1	1	1	2	1	1	1	1
FERUAU	2	1	1	1	1	1	1	3	1	1	1	1	1	1	1	2	1	1	1	1
HILPAR	1	2	2	2	2	3	1	23	2	2	1	1	2	1	1	1	1	23	1	1
HYMFLO	3	2	1	2	3	1	1	2	1	1	1	1	1	1	1	1	1	2	1	1
JOOAEQ	3	1	1	1	1	1	1	2	1	2	1	1	2	2	2	1	1	2	?	?
JOODIC	?	1	1	1	1	1	1	2	1	1	1	1	2	2	2	1	1	2	2	1

Table 2. Continued.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
JOODIE	2	1	2	1	1	1	1	1	3	3	23	2	1	12	?	1	1	2	2	2
JOOUMB	2	1	2	1	1	1	1	1	3	3	2	2	1	1	?	1	1	2	1	2
LADAMA	1	1	1	1	1	1	1	1	1	1	23	2	1	1	2	1	1	2	1	2
LADCAR	2	1	1	1	1	1	1	1	1	1	3	2	1	12	?	1	1	2	?	2
LADCRA	2	1	1	3	?	2	1	1	1	1	3	2	1	12	1	1	1	2	1	2
LADLAM	2	1	1	12	1	12	1	1	1	1	23	2	1	1	2	1	1	2	1	2
LADMAC	2	1	1	3	?	12	1	1	1	1	34	2	1	2	1	1	1	2	1	2
LADMOR	2	1	1	2	1	2	1	1	1	1	34	2	1	3	2	1	1	2	1	2
LADOBL	1	1	1	2	1	2	1	1	1	1	2	2	1	123	2	1	1	2	1	2
LADPAV	2	1	1	2	1	1	1	1	1	1	4	2	1	2	?	1	1	2	1	2
LADSER	2	1	1	2	1	2	1	1	1	1	4	2	1	1	?	1	1	2	1	2
LADUND	2	1	1	1	1	1	1	1	1	1	23	2	1	1	2	1	1	2	1	2
LUCINT	2	1	1	2	2	2	1	1	1	1	3	1	1	5	5	1	3	2	2	1
MACCUE	2	1	1	2	1	1	1	1	1	1	2	1	1	12	?	1	5	2	2	1
MACJAM	2	1	1	2	1	1	1	2	1	1	2	1	1	2	?	1	5	2	1	1
MACROS	1	1	1	2	1	12	1	2	1	1	2	1	1	123	?	1	5	2	2	1
MAGNEB	2	1	1	1	1	1	1	2	1	1	2	?	1	3	2	1	1	2	2	1
PIMGLO	2	1	1	1	1	2	1	2	1	1	1	1	1	1	3	1	1	1	1	2
REMAMA	2	2	1	12	3	2	1	2	4	1	2	2	1	2	?	1	1	2	1	2
REMFER	2	2	1	3	?	12	1	2	4	1	23	2	1	12	?	1	1	2	12	2
REMFIR	2	1	1	1	1	1	1	2	4	1	2	2	1	1	2	1	1	2	1	2
REMPAC	2	2	1	1	3	12	1	2	4	1	3	2	1	2	3	1	1	2	1	2
REMPED	2	1	1	2	1	2	1	2	1	1	12	2	1	12	?	1	1	2	1	2
REMPIL	2	1	1	1	1	1	1	2	4	1	12	2	1	1	?	1	1	2	2	2
REMPUR	2	1	1	1	1	2	1	2	1	1	2	2	1	3	2	1	1	2	1	2
REMVAU	2	2	1	1	3	2	1	2	4	1	3	2	1	2	3	1	1	2	?	2
STIGRA	2	1	1	1	1	2	2	2	1	1	1	1	1	1	2	1	1	2	1	1
STIOEL	2	1	1	2	1	2	2	2	1	1	2	?	1	2	2	1	1	2	1	2
WITSTA	1	1	1	2	2	1	1	1	5	1	12	1	1	3	?	2	3	2	2	3

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
JOODIE	1	1	1	1	1	2	2	2	1	2	1	1	1	2	1	2	1	1	1	1
JOOUMB	1	1	1	1	1	2	2	2	1	2	1	1	1	2	1	2	1	1	1	1
LADAMA	1	2	1	2	2	1	23	2	1	2	1	1	1	1	1	1	1	1	1	1
LADCAR	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
LADCRA	1	4	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
LADLAM	1	2	1	2	2	1	23	2	1	2	1	1	1	1	1	1	12	1	1	1
LADMAC	1	4	1	2	2	1	2	2	1	2	1	1	1	1	1	1	2	1	1	1
LADMOR	1	2	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
LADOBL	1	1	1	2	2	1	12	2	1	2	1	1	1	1	1	1	1	1	1	1
LADPAV	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
LADSER	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
LADUND	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	12	1	1	1
LUCINT	4	1	1	1	1	1	2	2	1	2	1	1	1	1	1	1	1	2	1	1
MACCUE	1	3	2	2	1	1	2	1	2	1	2	2	1	1	1	1	1	2	1	3
MACJAM	2	3	2	2	1	1	2	1	2	1	2	2	1	1	1	1	1	2	1	3
MACROS	12	3	2	2	1	1	2	1	2	1	2	2	1	1	1	1	1	2	1	3
MAGNEB	3	?	?	4	?	1	2	2	1	2	1	1	1	2	1	1	1	?	1	2
PIMGLO	3	1	1	4	1	1	2	1	1	2	1	1	1	2	1	1	1	1	1	1
REMAMA	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
REMFER	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	12	1	1
REMFIR	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	12	1	2
REMPAC	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	3	1	1	1
REMPED	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1
REMPIL	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	1	2	1	1
REMPUR	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	12	1	1	1	1
REMVAU	1	1	1	2	2	1	2	2	1	2	1	1	1	1	1	1	3	1	1	?
STIGRA	3	1	1	4	1	1	2	1	1	2	1	1	1	1	1	1	1	1	1	1
STIOEL	3	1	1	4	1	1	2	1	1	2	?	1	1	1	1	1	1	1	1	1
WITSTA	2	1	1	1	1	1	3	1	1	1	2	1	1	1	1	1	1	4	1	1

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
JOODIE	2	1	1	1	1	1	1	2	1	2	1	1	2	2	2	1	1	2	3	1
JOOUMB	3	1	1	1	1	1	1	2	1	1	1	1	2	2	2	1	1	2	?	?
LADAMA	1	1	1	1	1	1	1	3	1	1	2	1	2	2	2	1	1	2	?	?
LADCAR	1	1	2	1	1	1	12	3	1	2	2	1	2	2	2	1	1	2	1	1
LADCRA	1	1	2	1	1	1	1	3	1	2	1	1	2	2	2	1	2	2	1	1
LADLAM	1	1	1	1	1	1	1	23	1	12	2	1	2	2	23	1	?	3	1	1
LADMAC	1	1	2	1	1	1	1	3	1	2	2	1	2	2	2	1	2	2	1	1
LADMOR	1	1	2	1	1	1	2	23	1	2	2	1	2	2	2	1	1	2	1	1
LADOBL	1	1	1	1	1	1	1	3	1	2	1	1	2	2	2	1	1	2	1	1
LADPAV	1	1	1	1	1	1	1	3	1	2	1	1	2	2	2	1	1	3	1	1
LADSER	1	1	1	1	1	1	1	3	1	2	1	1	2	2	2	1	2	2	1	1
LADUND	1	1	2	1	1	1	2	3	1	2	2	1	2	2	2	1	1	3	1	1
LUCINT	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1
MACCUE	3	1	1	1	3	2	1	1	1	1	1	1	1	1	2	1	1	2	2	1
MACJAM	3	1	1	1	3	2	1	1	1	1	1	?	?	?	?	1	?	?	?	?
MACROS	3	1	1	1	3	2	1	1	1	2	1	1	1	1	12	1	1	2	2	1
MAGNEB	1	1	12	1	1	1	1	2	1	1	2	?	?	?	?	1	?	?	1	1
PIMGLO	1	1	1	1	1	1	1	2	1	2	2	1	2	2	2	1	1	2	1	1
REMAMA	1	1	1	1	1	1	1	2	1	2	2	1	2	2	2	1	1	2	1	1
REMFER	2	1	1	1	1	1	1	2	1	1	2	1	2	2	2	1	1	2	?	?
REMFIR	1	1	1	1	1	2	1	2	1	1	2	1	2	2	2	1	1	2	1	1
REMPAC	2	1	2	1	1	1	1	3	1	2	2	1	2	2	2	1	1	2	1	1
REMPED	1	1	1	1	1	1	3	2	1	1	2	1	2	2	2	1	1	?	1	1
REMPIL	1	1	1	1	1	1	1	12	1	1	2	1	2	2	12	1	1	2	1	1
REMPUR	1	1	1	1	1	1	3	12	1	2	2	?	?	?	?	1	?	?	?	?
REMVAU	?	?	1	1	1	1	1	3	1	2	1	1	2	2	2	1	1	3	1	1
STIGRA	1	1	1	1	1	1	1	1	1	2	2	?	?	?	?	1	?	?	1	1
STIOEL	1	1	1	1	1	1	1	1	1	2	2	1	2	2	2	1	2	2	1	1
WITSTA	2	1	1	2	1	2	1	1	1	2	1	1	1	1	2	1	1	1	1	1

(1994). However, according to accepted usage (e.g., Stearn, 1983) quinquncial is a special case of imbricate. In *Hillia*, *Cosmibuena*, and *Dolicholobium* the petals occur in two layers (typically three in the outer and two in the inner layer), being typically contorted in each layer. Aestivation is therefore coded as contorted in these taxa.

In *Cinchona* filament length differs considerably between brevistylous and macrostylous flower morphs. This is coded as a separate character state under character 28. In cases where only macrostylous flowers were seen this character is coded as missing data.

Stigma branch length (character 38) in heterostylous species was calculated on the basis of the macrostylous morph. In cases where only the brevistylous morph was observed, style length of the macrostylous morph was approximated to equal corolla tube length and the value was accepted if well separated from the class limit; if close to the class limit, the character was coded as missing data.

Pollen characters were discussed and documented in detail by Andersson (1993).

Results and Discussion

Throughout this discussion, clades will be designated by the numbers (Figs. 1 and 2) of the edges supporting them. Terminal taxa will be referred to by their traditional names, on which the acronyms used in the trees are based; a number of new names and combinations are introduced in the Synopsis. Only selected characters will be discussed here; complete apomorphy lists for the trees of Figures 1A and 2A are given in Appendix 2.

The Step I analysis run with unweighted characters resulted in 24 equally parsimonious trees with a length of 286 steps and an overall consistency index of 0.490. Successive reweighting gave nine equally parsimonious trees 289 steps long (calculated on the basis of unweighted characters) and with a consistency index of 0.484. Using Archie's (1989: 267) empirical formula, homoplasy excess ratio (HER) for the tree of Figure 1A was estimated at 0.587.

Both with weighted and unweighted characters, a basal dichotomy was found between clades 45 and 60 (Fig. 1A). Clade 45 is not supported by any unique and consistent apomorphies, but by three rare character states, long filaments (with parallelisms in *Pimentelia* and *Stilpnophyllum*), anthers fully exserted (with reversal in *Capirona*), and anthers button-shaped (with reversals in *Capirona* and *Wittmackanthus*). Clade 45 contains all genera (except *Schizocalyx*) referred to the Calycophylleae by Andersson & Persson (1991), plus three genera referred by them to the Cinchoneae: *Capirona*, *Ferdinandusa*, and *Macrocnemum*. *Macrocnemum* and *Capirona* are further nested in clade 45 by the presence of bristly hairs in the corolla tube and on the filaments, and by the interruption of the seed wing at the seed body. All are excluded from clade 60 by the lack of the apomorphic pollen characters foveolate exine and obtuse colpus ends. The conclusion seems well founded, therefore, that *Capirona*, *Ferdinandusa*, and *Macrocnemum* should be transferred to the Calycophylleae.

Calycophyllum in the traditional sense of, for example, Bacigalupo (1952) and Steyermark (1964, 1974) is divided between two clades, one (52) comprising the type species, *C. candidissimum*, and two closely related species, the other one (54) comprising the so-called "*Calycophyllum obovatum* group" (Steyermark, 1964). *Calycophyllum megistocaulum* (= *C. acreanum* Ducke), considered by Steyermark (1964) to be closely related to the "*C. obovatum*" group, is nested in clade 50 by virtue of its huge size, and small flowers with a campanulate corolla, but its position within the clade is not resolved. *Capirona* is nested in clade 50 on the basis of, among other things, huge size and presence of calycine semaphylls. It is nested in clade 51 also on the basis of the verruculose inside of its exotesta cells. *Capirona* is well characterized by several unique autapomorphies such as intrapetiolar stipules (stipules fused throughout intrapetiolarly and deeply divided interpetiolarly) and its unique exine structure (Andersson, 1993). This, taken together with the fact that clade 52 is likewise supported by several unique synapomorphies, makes it rather unattractive to merge *Capirona* with *Calycophyllum*. In consequence, *Calycophyllum* will have to be split into two genera, *Calycophyllum* s. str. (clade 52) and *Semaphyllanthe* (clade 54), described as a new genus in the Synopsis.

Calycophyllum s. str. (clade 52) is supported as monophyletic by three unique synapomorphies, i.e., sacciform bracts and bracteoles, calyx reduced to minute teeth, and filaments inserted at the sinuses. It is further supported by having corolla lobes imbricate in bud (parallelisms in *Wittmackanthus* and *Blepharidium*). *Semaphyllanthe* (clade 54) is supported only by more or less strongly homoplasious characters.

Alseis (clade 48) is well supported as monophyletic by five unique synapomorphies: protogynous flowers, corolla open in bud (reversed in at least one species excluded from this analysis), corolla

urceolate, puberulous style (reversed in several species excluded from this analysis), and placenta attached apically by a stipe. It is further supported by two rare character states that are more or less constant in the genus: subspicate (reduced thyrsiform) inflorescences (parallelisms in *Wittmackanthus* and *Hymenodictyon*), and filaments attached close to the corolla base (parallelism in *Calycophyllum intonsum*). Its sister-group relationship (clade 47) to the *Calycophyllum* group is supported by long recurved stigma branches (numerous reversals in the *Calycophyllum* group) and verruculose thickenings on the inner tangential wall of exotesta cells (reversal in *Calycophyllum intonsum*).

Ferdinandusa (clade 59) is supported as monophyletic by three unique synapomorphies: colleters placed in a continuous row along the base on the inside of the calyx, connective dorsally widened and reinforced, and lumina of the exine differentiated into two distinct size classes. It is further supported by one rare character state, large seed size (parallelisms in species of *Ladenbergia* and *Remijia*).

Macrocnemum (clade 57) is supported as monophyletic by three unique synapomorphies: corolla reduplicate in bud, corolla tube minutely papillose inside below the stamen attachments, and fruits loculicidally dehiscent throughout. It is further supported by three rare apomorphic character states: corolla lobes marginally minutely papillose (parallelisms in *Ladenbergia* and *Remijia*), thinnings in the corolla tube tissue running down from the sinuses to the stamen attachments (parallelism in *Cinchona*), and inner tangential wall of the exotesta cells with sheetlike secondary thickening with pores (parallelism in *Joosia dichotoma*).

The sister-group relationship between *Macrocnemum* and *Wittmackanthus* (clade 56) is supported only by homoplasious characters (reddish corolla, small seeds, foveolate exine). Steyermark & Kirkbride (1975) concluded that the closest relative of *Wittmackanthus* is *Calycophyllum*. However, *Wittmackanthus* lacks the critical synapomorphies of the *Calycophyllum* group, such as internally verruculose exotesta cells and curved style branches. Its position within the Calycophylleae seems rather uncertain.

Clade 60, which includes the remaining genera of the Cinchoneae and *Hillia*, is supported as monophyletic by a single unique synapomorphy in the runs of Step I, obtuse to truncate colpus ends (further transformed in *Blepharidium* and *Dolicholobium*, where pollen grains are porate).

Step II (Fig. 2) generated more than 1000 minimal trees in the run with unweighted characters, 234 steps long and with overall consistency indices of 0.490. After successive reweighting 569 minimal trees were found, 235 steps long (as calculated with unweighted characters) and with overall consistency indices of 0.579. The HER for the tree of Figure 2A was estimated at 0.485.

The differences in topology between the trees from Step I (Fig. 1) and those from Step II (Fig. 2) are striking. These differences seem to depend mainly on the fact that different outgroups are used. Since a closer outgroup is used in Step II, and since the ingroup contains a fuller selection of terminal taxa, edge lengths are shorter on the average. One is therefore justified in putting greater confidence in the results of Step II than those of Step I (see, e.g., Penny et al., 1992).

The ingroup (clade 44) is supported as monophyletic by four unique synapomorphies: included anthers, elongate to linear anthers, long connivent stigma branches, and obtuse to truncate colpus ends. It is further supported by two homoplastic character states: short filaments (reversed in *Pimentelia* and *Stilpnophyllum*) and foveolate tectum (reversed in *Hillia parasitica* and with further parallel transformations to psilate in *Blepharidium*, internally in *Cosmibuena grandiflora*, and in *Dolicholobium*).

Dolicholobium comes out as a basal clade (40) in Step II analyses, in contrast to those of Step I, where it comes out in a more distal position within a basal paraphyletic complex. Its position was variable also among the preliminary runs. This uncertainty may indicate that the assumption that it belongs to the Cinchoneae complex is faulty.

Both the run with unweighted and that with weighted characters indicate a basal dichotomy between one clade (45) comprising *Balmea*, *Blepharidium*, *Cosmibuena*, and *Hillia*, and another one (48) comprising *Cephalodendron*, *Cinchona*, *Dolicholobium*, *Joosia*, *Ladenbergia*, *Maguireocharis*, *Pimentelia*, *Remijia*, and *Stilpnophyllum*. This result is interpreted as support for the recognition of an extended tribe Hillieae (clade 45) that is the sister group of the tribe Cinchoneae (clade 48).

The tribe Hillieae (clade 45) is supported by one unique and consistent synapomorphy, fruit with an apical, beaklike prolongation, and also by the rare character state calyx shed in fruit (parallelisms in *Cephalodendron* and *Wittmackanthus*). The "central core" of the tribe (clade 46) is furthermore supported by presence of raphides. The phylogeny of this tribe (excluding *Blepharidium*) was studied in detail by Taylor (1994).

The tribe Cinchoneae (clade 48) is supported as monophyletic by an externally sericeous corolla (reversals in *Stilpnophyllum grandifolium* and *Maguireocharis*). If *Dolicholobium* is excluded (clade 49) it is supported by another two unique and consistent synapomorphies, corolla lobes valvate in bud and os of the pollen aperture poorly defined.

The genera *Maguireocharis*, *Pimentelia*, and *Stilpnophyllum*, together with *Cinchona amazonica*, form a monophyletic group (clade 77) that is the sister group of the rest (clade 50) of the Cinchoneae. Clade 77 is supported as monophyletic by two synapomorphies, corolla tube hirsute inside above stamens and corolla lobes hirsute all over adaxially. It is also set aside from the rest of the Cinchoneae by lacking the unique and consistent synapomorphy of clade 50, heterostylous flowers. The genus *Stilpnophyllum* (clade 80) is supported as monophyletic by its unusual leaf venation, with secondary veins very thin and closely set. Clade 79 is supported as monophyletic by a campanulate corolla, but since this character may easily be erroneously interpreted in very short-tubed flowers, it should not be overemphasized. Clade 78 is supported only by homoplastic characters. *Cinchona amazonica* must obviously be removed from the genus *Cinchona* (clade 71), in which it was supposedly originally placed based mainly on its acropetal fruit dehiscence. Recognizing that the resolution of clade 77 may not be particularly stable, I suggest that it be referred to a genus of its own, *Cinchonopsis*, formally described in the Synopsis.

The sister group relationship of *Cinchona* and *Joosia* (clade 70) is supported by the joint possession of reddish flowers, a character with several parallelisms and also reversals within *Joosia*. It is not supported in all solutions (Fig. 2C), and the two genera may come out as successive basal branchings of clade 50. Both genera are excluded from clade 51 by lack of two unique synapomorphies.

Joosia (clade 74) is supported as monophyletic by four unique and consistent synapomorphies: persistent stipules, predominantly monochasial partial inflorescences, corolla lobes with submarginal, showy appendages, and inner tangential exotesta walls with sheetlike thickening. It is further supported by absence of inflorescence bracts (parallelism in *Dolicholobium*) and hairy disk (parallelisms in *Cinchona calisaya* and *C. amazonica*). The segregation of *Joosia dichotoma* as a distinct subgenus, *Sectocalyx*, as suggested by Steyermark (1975), would leave the autonymous subgenus *Joosia* paraphyletic. Steyermark seems to have based his decision entirely on the occurrence in *J. dichotoma* of a unique autapomorphy, the spathaceous calyx.

Cinchona (clade 71) is supported as monophyletic by two unique and consistent synapomorphies, corolla lobe margins villous with thin-walled hairs and filaments distinctly different in length in brevi- and macrostylous flowers. It is further supported by the corolla tube with longitudinal thinnings (parallelism in *Macrocnemum*) and acropetal fruit dehiscence (consistent but with numerous parallelisms in *Ladenbergia* and *Remijia*). *Cinchona henleana*, traditionally (e.g., Standley, 1930, 1931a; Steyermark, 1974) included in *Cinchona* on the basis of its acropetally dehiscent fruits, is nested within the *Ladenbergia* clade and should be removed (see further below).

The sister-group relationship (clade 51) between *Ladenbergia* and *Remijia* is supported by two unique and consistent synapomorphies, corolla lobes with the apical part ridged and wedge-shaped and corolla lobe margins papillose. These two character states are conceivably functionally coupled, which may weaken the support for the clade.

Ladenbergia (clade 60) is supported as monophyletic by only two homoplasious characters, stipules fused at base (with several reversals and parallelisms, and a further transformation to fused throughout in clade 69) and large seeds (parallelism in clade 58). *Cinchona henleana* is nested within the *Ladenbergia* clade. It seems, intuitively, closely related to, if at all distinct from, two other species traditionally (e.g., Standley, 1930) treated here, *L. muzonensis* (Goudot) Standley and *L. hookeriana* (Wedd.) Standley. These two were separated as section *Muzonia* by Weddell (1849) and later elevated to generic rank by Osorio (1874). Judging from the present analysis, the recognition of *Muzonia*, as advocated by Sullivan & Steyermark (1983) would, however, force a fragmentation of the remaining part of *Ladenbergia* and seems, therefore, to be ill advised. The sections recognized by Weddell (1849) under the synonymous name *Cascarilla* seem to be in fair agreement with the present results, but are not really put to the test due to only partial correspondence in selection of taxa.

The genus *Remijia* (clade 52) is supported as monophyletic by one synapomorphy, terminal inflorescence absent, which is consistent but with numerous parallelisms. Clade 53, comprising all species except *R. pedunculata* and *R. purdieana* (clade 59), is further characterized by a unique synapomorphy, thyrsoid inflorescences (further transformed to capitate in *Cephalodendron globosum*). Clade 59 is supported as monophyletic by

marginally ciliate seed wings. The apically split capsule valves (fruit with partial loculicidal dehiscence), traditionally emphasized as a distinctive character of *Remijia*, is a highly homoplasious one. The genus *Cephalodendron* is nested within the *Remijia* clade and should be regarded as an apomorphic derivative of this genus; recognition of *Cephalodendron* would force the pulverization of *Remijia*.

SYNOPSIS

Tribe **Calycophylleae** L. Andersson & C. Persson, Pl. Syst. Evol. 178: 89. 1991. TYPE: *Calycophyllum* DC.

Trees or rarely shrubs. Raphides absent. Stipules usually clasping to contorted in bud, rarely held flat back-to-back, or calyptrate. Corolla lobes contorted, imbricate, open, or reduplicate in bud; filaments long, often bristly hirsute at base, anthers button-shaped to elongate, rarely linear, dorsifixed near middle, or basifixed; ovary multiovulate, with distinct placentae attached to septum by a lamella, or apically by a stipe. Pollen 3-colporate, the colpi with acute ends and os distinctly demarcated, or rarely 3-porate, exine usually reticulate, rarely ± foveolate. Fruit a usually septicidal capsule with a short loculicidal fissure at apex of valves, or (*Macrocnemum*) loculicidal. Seeds lenticular or planoconvex, with exotestal wing, hilum located centrally or marginally on seed body, or on wing, exotesta cells with bandlike thickenings on inner tangential wall, or (in *Macrocnemum*) the thickening sheetlike with pores.

Alseis Schott in Sprengel, Syst. Veg. 4(2): 404. 1827. TYPE: *Alseis floribunda* Schott in Sprengel.

Alseis is recognized by its subspicate (reduced thyrsiform) inflorescences, protogynous flowers with urceolate corolla having the lobes usually open in bud, and its pendent placentae. It is a neotropical genus supposed (Andersson, 1992) to comprise about 16 species. It is badly in need of taxonomic revision, however.

Calycophyllum DC., Prodr. 4: 367. 1830. TYPE: *Macrocnemum candidissimum* M. Vahl = *Calycophyllum candidissimum* (M. Vahl) DC.

Eukylista Benth., J. Bot. (Hooker) 5: 230. 1853. TYPE: *Eukylista spruceana* Benth. = *Calycophyllum spruceanum* (Benth.) J. D. Hook. ex Schumann.

Calycophyllum is recognized by its calyptrate stipules and sacciform bracts and bracteoles, calyx (except semaphylls) reduced to minute teeth (to sometimes totally obsolete), corolla lobes that are imbricate in bud, filaments inserted on the corolla at the sinuses, corolla tube bristly hirsute inside in the upper part, and glabrous filaments. It is a genus of three species distributed from northern Central America and the Greater Antilles to northern Argentina and Paraguay.

Capirona Spruce, J. Proc. Linn. Soc., Bot. 3: 200. 1859. TYPE: *Capirona decorticans* Spruce.

Monadelphanthus Karsten, Fl. Columb. 1: 67. 1860. TYPE: *Monadelphanthus floridus* Karsten = *Capirona decorticans* Spruce.
Loretoa Standley, Field Mus. Nat. Hist., Bot. Ser., 11: 221. 1936. TYPE: *Loretoa peruviana* Standley = *Capirona decorticans* Spruce.

Capirona is recognized by its intrapetiolar, persistent stipules, corolla with lobes contorted in bud and tube conspicuously plicate, filaments inserted near the base of the corolla tube and interconnected at base by a callosity, basifixed anthers, pollen with reticulate exine with very narrow muri, wide lumina, and numerous free, rodlike structures within the lumina, and by seeds with the hilum located on the wing. It is a monotypic genus (Andersson, 1994) distributed more or less throughout the Amazon basin and the Guianas.

Semaphyllanthe L. Andersson, gen. nov. TYPE: *Semaphyllanthe obovata* (Ducke) L. Andersson = *Calycophyllum obovatum* (Ducke) Ducke = *Warszewiczia obovata* Ducke.

Calycophyllo similis sed differt stipulis liberis, bracteis planis, calyce distincto, corolla aestivatione contorta, filamentis ad basin hirsutis, ad medium corollae tubo, vel basin versus affixis.

Trees. Leaves decussate; stipules interpetiolar, not fused at base, ± triangular, clasping in bud, caducous. Inflorescences terminal and lateral, cymose, clustered to form ± corymbose synflorescences at branch tips. Flowers small, homostylous, normally 4-merous; calyx ± cup-shaped, with distinct or indistinct lobes, some flowers usually (except in *S. megistocaula*) with one of the lobes expanded into a large semaphyll; corolla ± campanulate, tube ± equaling the lobes, or shorter, glabrous outside, densely bristly hirsute inside above stamen insertion, lobes contorted in bud, glabrous; stamens attached to corolla tube in central part or lower, filaments longer than anthers, bristly hirsute in lower part; anthers button-shaped, dorsifixed near middle, long exserted; stigma branches con-

nivent or widely recurved. Capsules septicidal with basipetal dehiscence, crowned by the persistent calyx. Seeds winged, hilum located ± centrally on seed body, exotesta cells with bandlike, ± verruculose secondary thickenings on inner tangential wall.

SPECIES INCLUDED:

Semaphyllanthe intonsa (Steyerm.) L. Andersson, comb. nov. Basionym: *Calycophyllum intonsum* Steyerm., Mem. New York Bot. Gard. 10(5): 186. 1963.

Semaphyllanthe megistocaula (K. Krause) L. Andersson, comb. nov. Basionym: *Remijia megistocaula* K. Krause, Bot. Jahrb. 40: 319. 1908.

Semaphyllanthe merumensis (Steyerm.) L. Andersson, comb. nov. Basionym: *Calycophyllum merumense* Steyerm., Mem. New York Bot. Gard. 23: 228. 1972.

Semaphyllanthe obovata (Ducke) L. Andersson, comb. nov. Basionym: *Warszewiczia obovata* Ducke, Notizbl. Bot. Gart. Berlin-Dahlem 11: 475. 1932.

Semaphyllanthe spectabilis (Steyerm.) L. Andersson, comb. nov. Basionym: *Calycophyllum spectabile* Steyerm., Mem. New York Bot. Gard. 10(5): 188. 1963.

Semaphyllanthe venezuelensis (Steyerm.) L. Andersson, comb. nov. Basionym: *Calycophyllum venezuelense* Steyerm., Mem. New York Bot. Gard. 10(5): 189. 1963.

Semaphyllanthe differs from *Calycophyllum* in having free (vs. calyptrate) stipules, plane (vs. saccate) bracteoles, a distinct tubular calyx (vs. one reduced to minute teeth), contorted (vs. imbricate) corolla aestivation, filaments inserted in center of corolla tube or lower (vs. at top), and filaments bristly hirsute toward base (vs. glabrous throughout). It is heavily centered on the Guiana shield and in the white sand campinas of the northern Amazon basin. Only the aberrant species *S. megistocaula* occurs outside of this area, in the southwestern Amazon. *Semaphyllanthe megistocaula* is included in the genus for practical reasons only: it shares the (plesiomorphic) diagnostic character states with the rest of the species, but is not reliably associated with them in the cladistic analysis.

Ferdinandusa Pohl, Pl. Bras. Icon. Descr. 2: 8. 1828. TYPE: Based on *Ferdinandea* Pohl.

Ferdinandea Pohl, Flora 10: 153. 1827, non *Ferdinanda* Lagasca. TYPE: Not designated.
Aspidanthera Benth., J. Bot. (Hooker) 3: 217. 1841. TYPE: *Aspidanthera rudgeoides* Benth. = *Ferdinandusa rudgeoides* (Benth.) Wedd.
Gomphosia Wedd., Ann. Sci. Nat. Bot., Sér. 3, 10: 14. 1848. TYPE: Not designated.

Ferdinandusa is recognized by its free, contorted stipules, its more or less cochlear corolla lobes that are contorted in bud, its anthers with a dorsally widened and reinforced connective, its reticulate exine with two distinct size classes of lumina, and its large seeds. It is a neotropical genus heavily centered in the Amazon basin and is supposed to contain ca. 25 species (Andersson, 1992). It seems badly in need of a taxonomic revision, however.

Macrocnemum P. Browne, Civ. Nat. Hist. Jamaica 165. 1756. TYPE: *Macrocnemum jamaicense* L.

Lasionema D. Don, London Edinburgh Philos. Mag. & J. Sci. 2: 377. 1833. TYPE: *Lasionema roseum* (Ruiz López & Pavón) D. Don = *Cinchona rosea* Ruiz López & Pavón = *Macrocnemum roseum* (Ruiz López & Pavón) Wedd.

Macrocnemum is recognized by its reduplicate corolla lobes which are minutely hirtellous along the margins, its corolla tube which is minutely papillose inside below the filament attachments and which has linear thinnings running down from the sinuses to the filament bases, its loculicidal capsules, and its small seeds with the hilum located terminally or subterminally on the basiscopic wing. It is distributed in southern Central America, on Jamaica, and in subandean South America south to central Bolivia. In spite of its having ca. 14 presently recognized species (Andersson, 1992), the number of good species is probably less than half a dozen.

Tribe **Hillieae** Bremek. ex S. Darwin, Taxon 25: 603. 1976. TYPE: *Hillia* Jacq.

Trees or shrubs, rarely subshrubs, often epiphytic or chasmophytic. Raphides usually present (absent in *Blepharidium*). Stipules clasping to contorted in bud, or (usually) held flat back-to-back. Corolla lobes contorted or (*Blepharidium*) imbricate in bud; filaments short, glabrous, anthers linear, dorsifixed near middle or near base, or basifixed; ovary multiovulate, with distinct placentae attached to septum by a lamella, ovules ascendingly imbricate. Pollen 3-colporate, colpi with obtuse ends, os distinctly demarcated, or pollen 3-porate, exine reticulate, or foveolate to ± psilate. Fruit a usually septicidal capsule, apical portion (above calyx scar) prolonged and ± beaklike, calyx shed after anthesis. Seeds lenticular or planoconvex to ellipsoid,

with exotestal wing (rudimentary in *Hillia*), in *Hillia* also with long exotestal trichomes, hilum located centrally or marginally on seed body, exotesta cells with bandlike thickenings on inner tangential wall.

The tribe Hillieae is recognized mainly by its large woody capsules with the apical portion more or less prolonged and beaklike in mature fruit, and from which the calyx is shed soon after anthesis. The central core is formed by the genera *Balmea*, *Cosmibuena*, and *Hillia*. These are further characterized by the presence of raphides and contorted aestivation.

Hillia Jacq., Enum. Syst. Pl. 3. 1760. TYPE: *Hillia parasitica* Jacq.

Ravnia Oersted, Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn 1852: 49. 1852. *Hillia* subg. *Ravnia* (Oersted) C.M. Taylor, Selbyana 11: 30. 1989.

Hillia is characterized primarily by its rudimentary seed wing and presence of long exotestal hairs. It comprises 24 species distributed from southern Mexico through Central America and the West Indies to southern Brazil and Bolivia. The genus was monographed by Taylor (1994).

Cosmibuena Ruiz López & Pavón, Fl. Peruv. 3: 2. 1802, nom. cons., non *Cosmibuena* Ruiz López & Pavón, Fl. Peruv. Prodr. 10. 1794, nom. rejic. TYPE: *Cosmibuena obtusifolia* Ruiz López & Pavón, typus cons. = *Cosmibuena grandiflora* (Ruiz López & Pavón) Rusby = *Cinchona grandiflora* Ruiz López & Pavón.

Buena Pohl, Pl. Bras. Icon. Descr. 1: 8. 1826, non Cav. 1800. TYPE: *Cosmibuena grandiflora* (Ruiz López & Pavón) Rusby = *Cinchona grandiflora* Ruiz López & Pavón.

Cosmibuena is distinguished from *Hillia* by its stipules being fused intrapetiolarly at base, its well-developed seed wing, and the absence of exotestal trichomes. It comprises four species and is distributed from southern Mexico to central Bolivia and southeastern Brazil. It was revised by Taylor (1992). Some of the cladistic analyses performed by Taylor (1994) indicate that *Cosmibuena* may be paraphyletic when *Hillia* is separated.

Balmea Martínez, Bull. Torrey Bot. Club 69: 438. 1942. TYPE: *Balmea stormae* Martínez.

Balmea differs from *Cosmibuena* mainly in having free stipules, a cymose, many-flowered inflorescence, and a deep red to dark purplish corolla. It is a monotypic genus with the single species, *B. stormae*, ranging from the state of Michoacán in central Mexico to northern Guatemala.

Blepharidium Standley, J. Wash. Acad. Sci. 8: 59. 1918. TYPE: *Blepharidium guatemalense* Standley.

Blepharidium agrees with other genera of the Hillieae in having large, woody capsules with a beaklike apical prolongation, and in having a caducous calyx. It differs from other members of the tribe in the absence of raphides, in having clasping stipules, corolla with imbricate aestivation, and anthers dorsifixed around the middle. It has been considered (Standley, 1940) to comprise two species, but *B. mexicanum* Standley is hardly specifically distinct from *B. guatemalense*. The genus is distributed from southern Mexico to northern Guatemala.

Tribe **Cinchoneae** DC., Ann. Mus. Natl. Hist. Nat. 9: 217. 1807, as "Cinchonaceae." TYPE: *Cinchona* L.

Trees or shrubs. Raphides absent. Stipules held flat back-to-back in bud, or (in species with verticillate leaves) held to form a cone and then marginally overlapping, free or intrapetiolarly fused at base, or calyptrate. Corolla lobes valvate in bud; filaments usually short or rather short, but often rather long in brevistylous morphs of heterostylous species, glabrous, anthers elongate to linear, dorsifixed near base; ovary multiovulate, with distinct placentae attached to septum by a lamella, ovules ascendingly imbricate. Pollen 3-colporate, colpi with obtuse to truncate ends, os poorly demarcated, exine foveolate. Fruit a septicidal capsule, apical portion not prolonged, calyx persistent. Seeds lenticular or planoconvex, with exotestal wing, hilum located centrally or marginally on seed body, exotesta cells with bandlike thickenings on inner tangential wall.

Pimentelia Wedd., Hist. Nat. Quinquinas 94. 1849. TYPE: *Pimentelia glomerata* Wedd.

Pimentelia is strongly reminiscent of *Stilpnophyllum* in having strongly "resinous" buds and young shoots, all inflorescences lateral, corolla strongly hairy inside in the upper part of the tube and all over the adaxial surface of the lobes, the hairs being coarse and thick-walled, and in having small capsules and small seeds. It differs, however, in having widely (vs. very narrowly) spaced lateral veins with a distinct tertiary venation in between.

Stilpnophyllum J. D. Hook., in Benth. & J. D. Hook., Gen. Pl. 2: 33. 1873. TYPE: *Stilpnophyllum lineatum* J. D. Hook.

Stilpnophyllum differs from *Pimentelia* mainly in leaf venation, having a large number (> 25 pairs) of fine, closely set secondary veins. It is a genus of four species (Andersson, 1994) distributed in the Andes of Ecuador and Peru.

Cinchonopsis L. Andersson, gen. nov. TYPE: *Cinchonopsis amazonica* (Standley) L. Andersson = *Cinchona amazonica* Standley.

A *Cinchona* differt flore parvo corolla alba campanulata tubo distali parte et lobis in tota superficiei grosse pubescentibus. A *Maguireocharide*, *Pimentelia*, et *Stilpnophyllo* differt praesentia inflorescentia terminali et capsula dehiscentia basifugi.

Trees. Leaves decussate; stipules interpetiolar, shortly fused across petiole at base, lingulate to triangular, held pressed back-to-back in bud, caducous. Inflorescences terminal and lateral, cymose. Flowers small, homostylous, normally 5-merous; calyx ± cup-shaped, with distinct lobes; corolla ± campanulate, white, tube ± equaling the lobes, or shorter, ± puberulous outside, densely bristly pubescent inside above stamen insertion, lobes valvate in bud, densely pubescent throughout on adaxial side with thick-walled hairs; stamens attached to corolla tube in central part, filaments ± equaling anthers, glabrous; anthers elongate, dorsifixed near base, with a small apical connective process, semi-exserted; stigma branches connivent. Capsules septicidal with acropetal dehiscence, crowned by the persistent calyx. Seeds winged, hilum located ± centrally on seed body, exotesta cells with bandlike, smooth secondary thickenings on inner tangential wall.

SPECIES INCLUDED:

Cinchonopsis amazonica (Standley) L. Andersson, comb. nov. Basionym: *Cinchona amazonica* Standley, Publ. Field Columbian Mus., Bot. Ser., 8: 334. 1931.

Standley (1931b) compared *Cinchona amazonica* to *C. micrantha* Ruiz López & Pavón and seems not to have been aware of the striking differences in floral structure between *C. amazonica* and other species of *Cinchona*. The decisive character for his placement of his new species was probably the acropetally dehiscent capsules. However, it differs from *Cinchona* in having small, homostylous flowers with a white, campanulate corolla, which is coarsely and densely pubescent inside in the distal part of the tube and throughout the adaxial surface of the lobes, and in the absence of thinnings on the corolla tube. *Cinchonopsis* differs from *Maguireocharis*, *Pimentelia*, and *Stilpnophyllum* in having terminal inflorescences and acropetally dehiscent capsules. The only species seems to be widely distributed in the central and western Amazon basin and has been recorded from Peru, Colombia, Brazil (Amazonas and Roraima), and Venezuela.

Joosia Karsten, Fl. Columb. 1: 9. 1858. TYPE: *Joosia umbellifera* Karsten.

Joosia is recognized by its persistent stipules, the showy marginal appendages on the corolla lobes, fruits with valves that are usually deeply split loculicidally and with the halves often conspicuously coiled. The inflorescences are predominantly monochasial but this is sometimes not readily seen when they are congested into headlike clusters. *Joosia* is a genus of seven species (Andersson, 1994) distributed from Panama along the Andes to northern Bolivia; it is heavily centered in the Ecuadorean Andes.

Cinchona L., Sp. Pl. 172. 1753. Gen. Pl. ed. 5 79. 1754. TYPE: *Cinchona officinalis* L.

Kinkina Adans., Fam. Pl. 2: 147. 1763. TYPE: Based on *Cinchona* L.
Cinchona [sect.] *Quinquina* Endl., Gen. Pl. 556. 1838. TYPE: *Cinchona officinalis* L. (lectotype, designated by Andersson, 1994).

Cinchona is recognized, above all, by the marginally villose corolla lobes, the hairs of which are thin-walled and somewhat widened distally. It is further characterized by having terminal inflorescences, reddish (pink to purple) flowers, by the corolla lobes not being wedge-shaped at the apex and by the linear thinnings present in the corolla tube. The acropetal dehiscence of the capsule previously used to distinguish *Cinchona* on the one hand and *Ladenbergia* and *Remijia* on the other (e.g., Schumann, 1891), seems to be a constant feature of *Cinchona* but is not a reliable diagnostic feature; it occurs in *Cinchonopsis* and many species of *Remijia* and *Ladenbergia* as well. *Cinchona* is a genus of 20 to 25 species distributed from Costa Rica and the coastal mountains of Venezuela along the Andes to central Bolivia; it is heavily centered in the Andes of Ecuador and northern Peru.

Ladenbergia Klotzsch in Hayne, Getreue Darstell. Gew. 14: tab. 15. 1846. TYPE: *Ladenbergia moritziana* Klotzsch in Hayne (lectotype, designated by Andersson, 1994).

Cinchona [sect.] *Cascarilla* Endl., Gen. Pl. 556. 1838. *Cascarilla* (Endl.) Wedd., Ann. Sci. Nat. Bot., Sér. 3, 10: 10. 1849. TYPE: *Ladenbergia macrocarpa* (M. Vahl) Klotzsch in Hayne = *Cinchona macrocarpa* M. Vahl (lectotype, designated by Andersson, 1994).

Cascarilla [sect.] *Pseudoquina* Wedd., Hist. Nat. Quinquinas 79. 1849. TYPE: Not designated.

Cascarilla [sect.] *Carua* Wedd., Hist. Nat. Quinquinas 83. 1849. TYPE: *Cascarilla carua* Wedd. = *Ladenbergia carua* (Wedd.) Standley (lectotype, designated by Andersson, 1994).

Cascarilla [sect.] *Calyptrata* Wedd., Hist. Nat. Quinquinas 89. 1849. TYPE: *Cascarilla macrocarpa* (M. Vahl) Wedd. = *Cinchona macrocarpa* M. Vahl = *Ladenbergia macrocarpa* (M. Vahl) Klotzsch in Hayne (lectotype, designated by Andersson, 1994).

Cascarilla [sect.] *Muzonia* Wedd., Hist. Nat. Quinquinas 89. 1849. *Muzonia* (Wedd.) N. Osorio, Estudio Quinas Est. Unid. Colombia, ed. 2, 24. 1874. TYPE: *Cascarilla muzonensis* (Goudot) Wedd. = *Cinchona muzonensis* Goudot = *Ladenbergia muzonensis* (Goudot) Standley.

Ladenbergia is recognized by the presence of terminal inflorescences, white flowers, corolla lobes with a wedge-shaped terminal portion which is minutely papillose, and, usually, by large seeds (≥ 11 mm long). It comprises ca. 40 species (Andersson, 1992) and has a wide distribution comprising southern Central America and most of humid tropical South America; it has conspicuous centers of diversity in the Guiana Highlands and the Northern Andes.

Remijia DC., Biblioth. Universelle Sci., Sci. Arts 41: 155. 1829. TYPE: *Remijia ferruginea* (A. St. Hil.) DC. = *Cinchona ferruginea* A. St. Hil. (lectotype, designated by Andersson, 1994).

Cephalodendron Steyerm., Mem. New York Bot. Gard. 23: 228. 1972. TYPE: *Cephalodendron globosum* Steyerm. (holotype).

Remijia is recognized by the absence of terminal inflorescences, usually thyrsiform inflorescences, white flowers, and by corolla lobes with the terminal part wedge-shaped and minutely papillose. It comprises ca. 40 presently recognized species (Andersson, 1992), but is partly in need of revision. It is widely distributed in tropical South America, but heavily centered on the western Guiana shield and adjacent parts of the Amazon basin.

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Appendix 1. Representative material examined.

Alseis eggersii Standley: *Dodson & Gentry 12466* (SEL, US); *Eggers 15738* (S). *A. floribunda* Schott in Spreng.: *Hatschbach 46834* (GB); *Regnell III.1524* (S). *A. yucatanensis* Standley: *Contreras 950* (S); *Lundell 17955* (S). *Balmea stormae* Martínez: *Hinton 7894* (F, MO, US); *Rzedowski 34593* (NY). *Blepharidium guatemalense* Standley: *Contreras 9383* (S); *Jones & Facey 3433* (NY). *Calycophyllum candidissimum* (M. Vahl) DC.: *Budowski 33* (NY); *Bunting 8250* (NY). *C. intonsum* Steyerm.: *Berg et al. in Prance 19493* (S); *Prance 3738* (S). *C. megistocaulum* (Krause) C. M. Taylor: *Croat 20768* (AAU); *Ducke 24414* (S). *C. multiflorum* Griseb.: *Malme 1070B, 3006* (S). *C. obovatum* (Ducke) Ducke: *Clark & Maquirino 7656* (GB); *Ducke 266* (NY). *C. spruceanum* (Benth.) Hook. f.: *Holm-Nielsen et al. 21056* (AAU); *Krukoff 5416* (S). *C. venezuelense* Steyerm.: *Maguire et al. 46789* (NY). *Capirona decorticans* Spruce: *Mori & Bolten 8413* (S); *Palacios 3225* (GB). *Cephalodendron globosum* Steyerm.: *Maguire 42467* (NY); *Nee 31171* (NY). *Cinchona amazonica* Standley: *Fernández & Sanoja 4964* (NY); *Fosberg 38838* (NY). *C. barbacoensis* Karsten: *Pittier 517* (US); *van der Werff et al. 12028* (GB, MO). *C. calisaya* Wedd.: *Krukoff 11374* (NY), *Rusby 2347* (NY). *C. lancifolia* Mutis: *Fosberg 19570* (S), *20057* (US). *C. macrocalyx* Pav. ex DC.: *Madsen 75475, 86152* (AAU). *C. officinalis* L.: *Eriksson et al. 222* (GB); *Hart 762* (A). *C. pubescens* Vahl: *Harling & Andersson 22436* (GB); *Prieto AP-18* (S). *Cosmibuena grandiflora* (Ruiz López & Pavón) Rusby: *Maguire et al. 53574* (S); *Krukoff 10418* (S). *Dolicholobium oblongifolium* A. Gray: *A. C. Smith 5109, 7296* (S). *Ferdinandusa elliptica* Pohl: *Hatschbach 44786* (GB). *F. goudotiana* Schumann: *Tillett et al. 45184* (S). *F. uaupensis* Spruce ex Schumann: *Berry 2168* (NY), *Clark 7245* (NY). *Hillia parasitica* Jacq.: *Shafer 3297* (S); *Tollsten 141* (GB). *Hymenodictyon floribundum* (Hochst. & Steud.) Robinson: *"M. R." 22857* (NY); *Taylor 3399* (S). *Joosia aequatoria* Steyerm.: *Harling & Andersson 23643* (GB). *J. dichotoma* (Ruiz López & Pavón) Karsten: *Schunke 1100* (NY), *5442* (S). *J. dielseana* Standley: *Steere 8026* (NY); *Steyermark 54262* (F). *J. umbellifera* Karsten: *Grant 9983* (S); *von Sneidern 5005* (S). *Ladenbergia amazonensis* Ducke: *Williams 14493* (NY). *L. carua* (Wedd.) Standley: *Krukoff 11021* (NY); *Rusby G-1* (NY). *L. crassifolia* (DC.) Standley: *Camp E-4793* (NY), *Prieto AP-26B* (NY). *L. lambertiana* (C. Martius) Klotzsch in Hayne: *Maguire 32864* (NY); *Tillett & Tillett 45276* (NY). *L. macrocarpa* (Vahl) Klotzsch in Hayne: *Camp E-4322* (NY); *Jaramillo 9352* (AAU). *L. moritziana* Klotzsch in Hayne: *Steyermark 56925* (NY). *L. oblongifolia* (Mutis) L. Andersson: *Camp E-43* (NY), *E-55* (S). *L. pavonii* (Lambert) Standley: *Dodson & Gentry 9716* (F); *Gentry et al. 54751* (GB). *L. sericophylla* Standley: *Asplund 18914* (S); *Fosberg 22474* (NY). *L. undata* Klotzsch in Hayne: *Steyermark 56107, 56178* (NY). *Luculia intermedia* Hutch.: *Rock 7032* (S); *Tsai 55159* (S). *Macrocnemum cuencanum* Standley: *Prieto CP-14* (NY); *Steyermark 52709* (F). *M. jamaicense* L.: *Proctor 9999* (NY). *M. roseum* (Ruiz López & Pavón) Wedd.: *Palacios 2768* (GB); *Williams 5468* (S). *Maguireocharis neblinae* Steyerm.: *Maguire 37346* (NY). *Pimentelia glomerata* Wedd.: *Hodge 6017* (US); *Solomon 9290* (GB). *Remijia amazonica* Schumann: *Krukoff 7967* (S); *Prance et al. 4923* (S). *R. ferruginea* (A. St.-Hil.) DC.: *Regnell III.90* (S). *R. firmula* (C. Martius) Wedd.: *Silva & Pinheiro 4207* (NY). *R. pacimonica* Standley: *Clark 6602* (NY). *R. pedunculata* (Karsten) Flückiger: *Fosberg & Fassett 21840* (S); *Jaramillo 6936* (GB). *R. pilosinervula* Steyerm.: *Pipoly et al. 7189* (NY); *Steyermark & Wurdack 713* (NY). *R. purdieana* Wedd.: *Fosberg & Fassett 21776* (NY). *R. vaupesiana* Steyerm.: *Frões 21225* (NY). *Stilpnophyllum grandifolium* L. Andersson: *Neill & Palacios 9538* (MO); *Palacios et al. 8563* (GB). *S. oellgaardii* L. Andersson: *Oellgaard 74918* (AAU). *Wittmackanthus stanleyanus* (R. M. Schomb.) Kuntze: *Gentry et al. 19052* (MO).

Appendix 2. Lists of apomorphies.

The two lists that follow use the following format: 7 3:1>>2 (0.500); 11:1>>3 [2](0.545). This should be interpreted thus: edge 7, character 3 transformed from state 1 to state 2 (consistency index 0.500); character 11 transformed from state 1 to state 3 [2 steps](consistency index 0.545). Double arrows (>>) indicate that the change is unambiguous, single arrows (>) that it is ambiguous. The number of steps is given only when distinct from 1. An asterisk indicates that this character was excluded during search in this particular run.

Figure 1A.—2 1:2>>1 (0.125). 4 16:1>>2 (0.250); 36:1>>2 (0.333); 60:2>>1 (0.500). 5 46:1>>2 (0.500). 7 3:1>>2 (0.500); 11:1>>3 [2](0.545); 16: 1>>2 (0.250); 30:1>>3 [2](0.429); 31:2>>1 (0.500); 32:2>>1 (0.333); 41:2>>1 (0.200); 45:1>>3 (0.333); 50:1>>2 (0.091). 8 19:1>>2 (0.300); 27:2>>1 (0.500); 34:1>>2 (0.200); 50:1>>2 (0.091). 10 1:1>>2 (0.125). 12 21:2>>1 (0.857). 13 19:2>>1 (0.300). 14 1:2>>1 (0.125); 50:1>>2 (0.091). 15 1:2>>1 (0.125); 9:1>>5 (0.625); 16:1>>2 (0.250); 20:1>>3 (0.500); 27:2>>3 (0.500); 29:2>1 (0.333); 32:2>>1 (0.333); 38:2>>4 (0.571); 44:1>>2 (0.250); 50:1>>2 (0.091). 16 1:2>>1 (0.125); 11:2>>3 (0.545); 51:1>>2 (0.222). 17 1:2>>1 (0.200). 18 14: 3>>1 (0.583). 19 19:1>>2 (0.300); 41:1>2 (0.200); 50:2>>1 (0.091). 20 4:1>>2 (0.400); 11:3>>4 (0.545); 14:3>>2 (0.583); 45:1>>3 (0.333); 52:1>>2 (0.750); 58:2>>3 (0.375). 21 1:2>>1 (0.125); 5:1>>2 (0.500); 17:2>>3 (0.571); 30:2>>1 (0.429); 41:1>2 (0.200); 55:2>>3 (0.800). 22 9:1>>2 (0.625); 21:1>>4 (0.857); 41:1>>2 (0.200); 44:1>>2 (0.250). 23 14:2>>3 (0.583); 22:1>>2 (1.000); *43:1>>2 (0.600); *47:1>>2 (0.667); 51:1>2 (0.222). 24 6:2>>1 (0.500); 58:2>>3 (0.375). 25 1:2>>1 (0.125). 26 *2:1>>2 (1.000); 5:1>>3 (0.500); 9:1>>4 (0.625). 27 *47:1>>3 (0.667). 28 9:1>>4 (0.625); 46:1>>2 (0.500). 29 6:1>>2 (0.500); *43: 1>>2 (0.600); 51:1>2 (0.222). 30 6:1>>3 (0.500). 32 14:1>>4 (0.583). 33 19:1>>2 (0.300); 50:1>>2 (0.091). 34 41:2>3 (0.200). 35 4:1>>2 (0.400); 8:2>>1 (0.333); 36:1>>2 (0.333); 51:2>1 (0.222). 36 15:2>>3 (0.500); 34:1>>2 (0.200). 37 20:2>>1 (0.500). 38 4:1>>2 (0.400); 11:1>>2 (0.545); 14: 1>>2 (0.583). 39 19:1>>2 (0.300); 20:2>>1 (0.500); 34:1>>2 (0.200). 40 4:1>>2 (0.400); 10:1>>3 (0.667); 14:1>>2 (0.583); 30:2>>3 (0.429); 58:2>>3 (0.375). 41 9:1>>6 (0.625); 11:3>>4 (0.545); 14: 3>>5 (0.583); 30:2>>3 (0.429); *43:1>>2 (0.600); 45:1>>2 (0.333); 46:1>>3 (0.500). 42 9:1>>5 (0.625); 11:2>>1 (0.545); 17:2>>1 (0.571); 18:2>>3 (0.400); 19:1>>2 (0.300); 33:1>>2 (0.500); 34:1>>2

(0.200); 38:2>>4 (0.571); 40:1>>3 (0.333); 41:1>3 (0.200); 45:1>>3 (0.333). 45 28:2>>1 (0.667); 30: 2>>1 (0.429); 31:1>>2 (0.500); 32:1>>2 (0.333); 41:1>2 (0.200); 42:2>>1 (0.500); 44:2>>1 (0.250); 58:2>>1 (0.375). 46 21:1>>2 (0.857); 29:1>2 (0.333); 46:1>2 (0.500). 47 11:2>>1 (0.545); 18: 2>1 (0.400); 38:2>1 (0.571); 60:1>>2 (0.500). 48 9:1>>5 (0.625); 13: 1>>2 (1.000); 17:2>>4 (0.571); 18:1>3 (0.400); 27:2>>1 (0.500); 37:1>>2 (1.000); 38:1>3 (0.571); 39:1>>2 (1.000); 45:1>>3 (0.333). 49 58:1>>2 (0.375). 50 1:2>>1 (0.125); 14:3>>2 (0.583); 46:2>1 (0.500). 51 4:1>>3 [2](0.400); 6:1>>2 (0.500); 15:2>4 (0.500). 52 10:1>>2 (0.667); 14:2>>6 (0.583); 17:2>>3 (0.571); 27:2>>3 (0.500); 29:2>>1 (0.333). 53 6:2>>3 (0.500); 38:1>>3 (0.571); 58:1>>2 (0.375). 54 14:2>>1 (0.583); 16: 1>>2 (0.250). 55 *47:1>>2 (0.667); 51:1>>2 (0.222). 56 4:1>>2 (0.400); 17:2>3 (0.571); 19:1>>2 (0.300); 48:2>>1 (0.714); 55:1>>2 (0.800). 57 5:2>>1 (0.500); 14:3>>2 (0.583); 17:3>5 (0.571); 22:1>>3 (1.000); 23:1>>2 (0.500); 24:1>>2 (0.750); 40:1>>3 (0.333); 41:2>>3 (0.200); 45:1>>3 (0.333); 58:1>>2 (0.375); 59:1>>2 (0.667). 58 8:1>>2 (0.333). 59 15:2>>1 (0.500); 35:1>>2 (1.000); 48: 2>>3 (0.714); 56:1>>2 (1.000). 60 5:2>>1 (0.500); 11:2>>3 (0.545); 27:2>>3 (0.500); 49:1>2 (0.500); 50:1>>2 (0.091); 53:1>>2 (1.000). 61 55:1>>2 (0.800). 62 15:2>3 (0.500); 33:1>>2 (0.500). 63 8:1>>2 (0.333); 14:3>1 (0.583); 38:2>>1 (0.571); 49:2>1 (0.500); 52:1>3 (0.750). 64 20:1>>2 (0.500); 27:3>>2 (0.500); 42:2>>1 (0.500); 44:2>>1 (0.250). 65 11:3>>2 (0.545); 17:2>>1 (0.571); 40:1>2 (0.333); 50:2>1 (0.091); 51:1>2 (0.222); 52:3>1 (0.750); 54:1>>2 (1.000). 66 12:1>>2 (1.000). 67 24:1>>2 (0.750); 25:1>>2 (1.000). 68 4:1>2 (0.400); 14:1>>2 (0.583); 40:2>1 (0.333). 69 6:1>>2 (0.500). 70 50:1>>2 (0.091). 71 8:2>>1 (0.333); 48:2>>3 (0.714); 51:2>1 (0.222). 72 11:2>>4 [2](0.545). 73 8:2>>1 (0.333); 15:2>3 (0.500); 51:2>1 (0.222). 74 19:1>>2 (0.300); 23:1>>2 (0.500); 24:1>>3 (0.750); 28:2>>3 (0.667). 75 50:1>>2 (0.091). 76 3:1>>2 (0.500); 9:1>>3 (0.625); 10:1>>3 (0.667); 26:1>>2 (1.000); 36:1>>2 (0.333); 40:2>1 (0.333); 41:1>2 (0.200); 59:1>2 (0.667). 77 34:1>>2 (0.200); 59: 2>3 (0.667). 78 14:1>3 (0.583); 21:1>>3 (0.857); 24:1>>4 (0.750). 79 6:1>>2 (0.500); 11:2>>1 (0.545); 18:2>1 (0.400); 50:1>2 (0.091). 80 14:3>1 (0.583); 28:2>>1 (0.667); 40:2>1 (0.333). 81 7:1>>2 (1.000); 18:1>2 (0.400); 48:2>>1 (0.714); *57:1>2 (1.000).

Figure 2A.—1 *14:2>3 (0.517); 19:1>>2 (0.375); 50:2>>1 (0.273). 2 4:1>>2 (0.375); 45:2>3 (0.667); 52:1>>2 (0.667). 3 9:1>>6 (1.000); *14:2>5 (0.517); *33:2>1 (0.500); 43:1>>2 (0.500); *46:1>>3 (0.667); 55:2>>1 (0.857). 4 1:2>1 (0.250); 5:1>>2 (0.667); 7:2>>3 (1.000); 30:2>1 (0.600); 52:1>>3 (0.667); 55:2>>3 (0.857). 5 9:4>>2 (1.000); *14: 1>>2 (0.517); 21:1>>4 (1.000); 41:1>>2 (0.286); 44:1>>2 (0.500). 6 8:1>>2 (0.600). 7 40:1>>2 (0.333); *46:1>>2 (0.667). 9 4:1>>3 [2](0.375); 50: 2>>1 (0.273). 10 43:1>>2 (0.500). 11 51:2>>1 (0.200); 58:2>>3 (0.375). 12 4:1>>2 (0.375); 50: 2>>1 (0.273). 13 *14:1>>3 (0.517). 14 1:2>>1 (0.250). 15 58:2>>3 (0.375). 16 *14:1>>2 (0.517); 15:2>>4 (0.429); 40:1>>2 (0.333). 17 *14:1>>2 (0.517); 58:2>>3 (0.375). 18 6:1>2 (0.600); 57:1>>2 (0.333). 20 58:2>>3 (0.375). 21 *14:1>>3 (0.517); 22:1>>2 (0.750). 22 51:2>1 (0.200). 23 37:1>>2 (1.000). 24 1:2>>1 (0.250). 25 *14:1>>2 (0.517); 48:2>>3 (0.692). 26 36:1>>2 (0.500). 28 *14:1>>2 (0.517). 29 43:1>>2 (0.500). 31 11:2>>3 (0.739). 32 *14:1>>4 (0.517). 33 34:1>>2 (0.250). 34 34: 1>>2 (0.250); *59:2>3 (0.667). 35 4:1>>2 (0.375); 8:2>>1 (0.333); *14:1>3 (0.517); 28:1>2 (0.500); 36:1>>2 (0.500); 40:1>>2 (0.333); 51:2>>1 (0.200). 36 34:1>>2 (0.250). 37 20:2>>1 (0.333). 38 4:1>>2 (0.375); 11:1>2 (0.739); *14:1>2 (0.517). 39 *14: 1>3 (0.517); 19:1>>2 (0.375); 20:2>>1 (0.333); 34: 1>>2 (0.250); 40:1>>2 (0.333); 50:2>>1 (0.273). 40 4:1>>2 (0.375); 10:1>>3 (0.500); *14:1>2 (0.517); 30:2>>3 (0.600); 52:1>>3 (0.667); 58:2>>3 (0.375). 41 5:1>>2 (0.667); 8:2>>1 (0.333); *14: 1>3 (0.517); *35:1>>2 (1.000); 48:2>>3 (0.692); 50:2>>1 (0.273); 51:1>>2 (0.200); *56:1>>2 (1.000); 58:2>>1 (0.375). 42 4:1>>2 (0.375); 11: 3>>2 (0.739); 17:2>>5 (1.000); 19:1>>2 (0.375); 22:1>>3 (0.750); 23:1>>2 (0.500); 24:1>>2 (0.750); *29: (1.000); 40:1>>3 (0.333); 41:2>>3 (0.286); 45: 1>>3 (0.667); *46:1>>2 (0.667); 48:2>>1 (0.692); *59:1>>2 (0.667). 44 1:1>2 (0.250); 15:1>2 (0.429); 28:1>>2 (0.500); 30:1>2 (0.600); *31:2>>1 (1.000); *32:2>>1 (1.000); 38:2>1 (0.600); 53:1>>2 (1.000); 55:1>2 (0.857). 45 27:2>>3 (1.000); 42:1>>2 (1.000); 44:1>>2 (0.500). 46 8:2>>1 (0.333); *14: 1>2 (0.517); 15:2>3 (0.429); *33:1>2 (0.500); 38: 1>2 (0.600); 49:1>>2 (1.000). 47 11:3>>4 (0.739); 30:2>3 (0.600); 41:2>>1 (0.286); 45:1>2 (0.667); 58:2>3 (0.375). 48 20:1>>2 (0.333); 41:2>>1 (0.286). 49 1:3>>2 (0.739); 17:2>>1 (1.000); 54: 1>>2 (1.000). 50 8:2>1 (0.333); 12:1>>2 (1.000). 51 6:1>>2 (0.600); 24:1>>2 (0.750); 25:1>>2 (1.000). 52 8:1>2 (0.333); 51:1>>2 (0.200). 53 9:1>>4 (1.000). 54 6:2>>1 (0.600); 50:2>>1 (0.273). 55 19:1>2 (0.375). 56 2:1>>2 (1.000); 5:1>>3 (0.667); *14:1>>2 (0.517); 15:2>3 (0.429). 57 11:2>3 (0.739); 41:1>>2 (0.286). 58 37:1>>3 (1.000); 48:2>>3 (0.692). 59 47:1>>3 (1.000). 60 4:1>>2 (0.375); 48:2>>3 (0.692). 61 11:2>>3 (0.739). 62 6:2>1 (0.600). 63 50:2>>1 (0.273). 64 4:2>1 (0.375); 22:1>>2 (0.750); 51:1>>2 (0.200). 65 11:3>>4 (0.739). 66 43:1>>2 (0.500); 51:1>2 (0.200). 67 47:1>>2 (1.000). 68 4:2>>1 (0.375); 6:2>>1 (0.600). 69 4:2>>3 (0.375); *14:1>2 (0.517). 15:2>>1 (0.429); 22:1>>4 (0.750); 57:1>>2 (0.333). 70 15:2>3 (0.429); 19:1>>2 (0.375). 71 23:1>>2 (0.500); 24:1>>3 (0.750); 28:2>>3 (0.500); 40:1>>2 (0.333). 72 6:1>>3 (0.600). 73 6:1>2 (0.600); 50: 2>>1 (0.273); 51:1>>2 (0.200). 74 3:1>>2 (1.000); 9:1>>3 (1.000); 10:1>>3 (0.500); 26:1>>2 (1.000); 36:1>>2 (0.500); 41:1>2 (0.286); *59:1>2 (0.667). 75 41:2>3 (0.286). 76 19:2>>1 (0.375); 50:2>>1 (0.273). 77 21:1>>3 (1.000); 24:1>>4 (0.750); 51: 1>>2 (0.200). 78 6:1>>2 (0.600); 11:2>1 (0.739); 28:2>1 (0.500). 79 15:2>3 (0.429); 18:2>>1 (1.000). 80 7:1>>2 (1.000); 48:2>>1 (0.692); 57:1>2 (0.333).

PHYLOGENY OF THE RUBIACEAE–RUBIOIDEAE, IN PARTICULAR THE TRIBE RUBIEAE: EVIDENCE FROM A NON-CODING CHLOROPLAST DNA SEQUENCE[1]

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ABSTRACT

A phylogenetic analysis of 39 species of the tribe Rubieae and of 15 taxa belonging to 12 other tribes of Rubiaceae has been performed using the DNA sequence of the chloroplast *atp*B-*rbc*L intergene region. The subfamily Rubioideae may be characterized as a monophylum, i.e., by a characteristic 204-bp deletion, shared by the representative tribes Coccocypseleae, Psychotrieae, Hedyotideae (paraphyletically linked to Spermacoceae), Anthospermeae, Theligoneae, and Paederieae, which, in this order, step-wise approach the advanced Rubieae. This tribe is clearly monophyletic and characterized by an additional 50-bp deletion. Five clades can be recognized within Rubieae, which mostly corroborate, but also partly contradict, traditional groupings (i.e., *Galium* and *Asperula* appear to be of polyphyletic origin); some of these results may have taxonomic implications.

By comparing the nucleotide sequences of a well-defined chloroplast intergene region among different genera and species, we hope to contribute to the reconstruction of the phylogeny of the tribe Rubieae. This tribe belongs to the huge, mostly woody, tropical and subtropical family Rubiaceae, one of the largest of all angiosperm families, with about 637 genera and more than 10,000 species (Mabberley, 1987). In contrast, the Rubieae, containing predominantly perennial to annual herbs with pseudowhorls of leaves and leaflike stipules, and composed of about 13 genera (Ehrendorfer, unpublished), is centered in temperate and tropical-mountain regions. The evolutionary radiation of the Rubieae has resulted in a worldwide distribution, but apparently is relatively recent. A survey of the literature (Muller, 1981) indicates that the first fossil pollen records of the Rubieae are from the Upper Miocene for the genus *Rubia* (Van Campo, 1976) and the Pliocene for the genus *Galium* (Menke, 1976). Because the tribe Rubieae is supposedly of relatively recent origin, instead of the widely used *rbc*L sequence, a non-coding sequence of the chloroplast DNA, the *atp*B-*rbc*L spacer, was chosen. We thought that this sequence, being under lower selective constraints, would exhibit higher variability between the different studied genera and species. This assumption has been proposed by Gielly & Taberlet (1994) for a study of the genus *Gentiana*, using other non-coding cpDNA sequences.

By comparison of this non-coding sequence (Manen et al., 1994), we had presented a phylogenetic analysis of 25 species of the tribe Rubieae, using six tropical genera from other tribes of Rubiaceae as outgroups. In a separate paper (Ehrendorfer et al., 1994) we briefly discussed the relationships among these outgroup tribes and their affinities with the tribe Rubieae. In this paper we extend these data by adding the sequences of 23 more Rubiaceae taxa. This allows a precise and useful delimitation of the subfamily Rubioideae by a very characteristic deletion, as well as, in spite of the addition of many taxa, the confirmation of the previously suggested general traits of the phylogeny of the tribe Rubieae and of the polyphyly of the genera *Galium* and *Asperula*.

MATERIALS AND METHODS

The list of the Rubiaceae taxa studied so far is shown in Table 1. It represents 8 genera, 39 spe-

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Table 1. Sources of cpDNA (fresh leaves) from the Rubiaceae species: 64 populations belonging to 54 taxa. * The numbers correspond to the numbers that appear in the phylogenetic trees (see Figures 1 and 2). ** Collectors: JEA = Jeanmonod, Daniel; MAN = Manen, Jean-François; NAT = Natali, Alessandro; PAL = Palese, Raoul; ROG = Roguet, Didier; THI = Thiébaud, Marc-André; ZEL = Zelweger, Catherine; E = Ehrendorfer, Friedrich; MK = Kiehn, Michael. The number is the collector number; all vouchers have been deposited in the herbaria of Geneva (G) or Universität Wien (WU). JBG + number stands for the living collection number in the Botanical Garden of Geneva. HBV + number stands for the living collection number in the Botanical Garden of Vienna.

No.*	Species	Locality	Voucher information**
Rubieae			
33	*Rubia peregrina* L.	Elba Island	NAT & THI/N56965
11	*R. tinctorum* L.	Geneva, Bot. Gard.	JBG 916690
02	*Sherardia arvensis* L.	Corsica, St. Petrone	JEA & NAT/J5048
05	*Sherardia arvensis* L.	Geneva	NAT & MAN/007
29	*Crucianella angustifolia* L.	Corsica, Francardo	JEA & NAT/J5044
35	*Phuopsis stylosa* (Trin.) Jackson	Geneva, Bot. Gard.	JBG 916798
19	*Asperula laevigata* L.	Elba Island	NAT & THI/s.n.
01	*A. tinctoria* L.	Geneva, Bot. Gard.	JBG 780680
51	*A. hirta* Ramond	Geneva, Bot. Gard.	JBG 814140/0 M
69	*A. chlorantha* Boiss. & Heldr.	Greece, Epirus	E 930413-4401
81	*A. cynanchica* L.	Geneva, Bot. Gard.	JBG 861771/0
82	*A. gussonii* Boiss.	Geneva, Bot. Gard.	JBG 783214/0
77	*A. purpurea* (L.) Ehrend.	Italy, Alpi Apuane	NAT & MAN/011
34	*Cruciata laevipes* Opiz	Corsica, Radicale	JEA, NAT, PAL/J4198
22	*C. glabra* (L.) Ehrend.	Elba Island	NAT & THI/N57761
32	*Valantia muralis* L.	Corsica, Pigno	JEA & NAT/s.n.
12	*Galium mollugo* L.	Corsica, Calvi	JEA, NAT, ZEL/s.n.
03	*G. album* L.	Corsica, Solenzara	JEA, NAT, PAL/s.n.
25	*G. album* L.	Corsica, S. Michelle	JEA & NAT/J4969
13	*G. album* L.	Geneva, Lullier	NAT & MAN/008
30	*G. album* L.	Corsica, Miomo	JEA & NAT/J4935
26	*G. album* L.	Corsica, St. Florent	JEA & NAT/J4963
31	*G. corrudifolium* Vill.	Elba Island	NAT & THI/N56941
23	*G. verum* L.	Geneva, Lullier	NAT & MAN/009
18	*G. lucidum* All.	Elba Island	NAT & THI/N56959
08	*G. lucidum* All.	Corsica, Strette	JEA & NAT/J4964
21	*G. aetnicum* Biv.	Capraia Island	NAT & THI/N57944
09	*G. corsicum* Spreng.	Corsica, Col St. Jean	JEA & NAT/J4931
07	*G. scabrum* L.	Corsica, Porto	JEA & ROG/J4961
20	*G. scabrum* L.	Elba Island	NAT & THI/N56964
16	*G. scabrum* L.	Elba Island	NAT & THI/N57753
17	*G. rotundifolium* L.	Corsica, Loreto di Casinca	JEA & NAT/J4979
06	*G. elongatum* C. Presl	Corsica, St. Florent	JEA & NAT/J4966
14	*G. palustre* L.	Geneva	NAT & MAN/s.n.
04	*G. divaricatum* Lam.	Corsica, Ajaccio	JEA, NAT, ZEL/J3394
10	*G. parisiense* L.	Corsica, Radicale	JEA, NAT, PAL/J4186
24	*G. verrucosum* Hudson	Corsica, Bonifacio	JEA, PAL, ROG/J3980
15	*G. aparine* L.	Corsica, Pietrabugno	JEA & NAT/s.n.
27	*G. aparine* L.	Corsica, St. Petrone	JEA & NAT/J5017
28	*G. aparine* L.	Corsica, Ponte Leccia	JEA & NAT/s.n.
52	*G. baillonii* Brandza	Romania, Prov. Arges	E 890821-3001
54	*G. murale* (L.) All.	Ionian Islands	E 930409-2502
55	*G. intricatum* Margot & Reuter	Ionian Islands	E 930409-2501
70	*G. perralderii* Coss.	Algeria, Kabylie	E 930626-1001
75	*G. rubioides* L.	Geneva, Bot. Gard.	NAT & MAN/013
76	*G. tricornutum* Dandy	Geneva, Bot. Gard.	NAT & MAN/014
79	*G. boreale* L.	Geneva, Bot. Gard.	JBG 814159/0
80	*G. odoratum* (L.) Scop.	Geneva, Versoix	NAT & MAN/016
45	*G. septentrionale* Roem. & Schult. (=*G. boreale* L. ?)	USA, Colorado	NAT & MAN/017

Table 1. Continued.

No.*	Species	Locality	Voucher information**
Paederieae			
53	*Putoria calabrica* (L. f.) DC.	Ionian Islands	E 930416-6201
Theligoneae			
67	*Theligonum cynocrambe* L.	Ionian Islands	E 930416-6201
Anthospermeae			
59	*Coprosma montana* Hillebr.	Hawaiian Islands, Maui	MK-910114-1/1
60	*C. ernodeoides* A. Gray	Hawaiian Islands, Maui	MK-910114-1/2
Spermacoceae			
65	*Spermacoce assurgens* Ruiz & Pavón	Costa Rica, Guanacaste	MK-880317-2/2
Psychotrieae			
42	*Hydnophytum formicarum* Jack	Geneva, Bot. Gard.	NAT & MAN/001
44	*Psychotria bacteriophila* Valet.	Geneva, Bot. Gard.	NAT & MAN/002
Hedyotideae			
41	*Bouvardia glaberrima* Engelm.	Geneva, Bot. Gard.	NAT & MAN/003
47	*Pentas lanceolata* (Forssk.) Deflers	Geneva, Bot. Gard.	NAT & MAN/004
Coccocypseleae			
64	*Coccocypselum* sp.	French-Guayana	HBV RR-91-19
Ophiorrhizeae			
63	*Ophiorrhiza* sp.	Indonesia, Sumatra	HBV RR-89-6
Hillieae			
72	*Hillia valerii* Standl. [=*Cosmibuena valerii* (Standl.) C.M. Taylor]	Costa Rica, Heredia	MK-880331-1/2
Hamelieae			
73	*Hoffmannia refulgens* Hemsl.	Mexico, Oaxaca	HBV RR-91-17
Coffeeae			
37	*Coffea arabica* L.	Geneva, Bot. Gard.	NAT & MAN/005
Pavetteae			
38	*Ixora parviflora* Vahl	Geneva, Bot. Gard.	NAT & MAN/006

cies, and 49 samples for the tribe Rubieae. To evaluate possible infraspecific variations, from several species different populations coming from various regions have been studied, including three different populations of the polymorphic *Galium aparine*, three populations of *Galium scabrum*, two populations of *Galium lucidum*, five populations of *Galium album*, and two populations of *Sherardia arvensis*. When variations (in fact very few) have been detected, the variable taxa have been included in the analysis. To delimit the tribe Rubieae and to test its monophyly, outgroup species, mainly from the subfamily Rubioideae, have been included in the analysis. Altogether, this analysis is based on 64 samples representing 54 species of Rubiaceae. All of these sequences are registered in the EMBL data bank under the accession codes X76457 to X76481 and X81669 to X81690.

Plant material was field-collected, obtained from seed that we grew in the greenhouses of the Geneva Botanical Garden, or taken from the living collections of the Botanical Gardens in Geneva and Vienna. Following the recommendations of Goldblatt et al. (1992), for each analyzed sample a voucher specimen has been prepared and deposited in the Geneva (G) or Vienna University (WU) herbaria.

The sequence we have chosen for this analysis is the intergene sequence located between the *rbc*L and the *atp*B gene of the plastid genome (see Manen et al., 1994, for more details). The *rbc*L and *atp*B genes are on opposite DNA strands. Thus, their intergene sequence contains the promoters of the two genes. We have studied this intergene region in a wide range of dicotyledonous orders and found that the *atp*B gene is regulated by two different promoters, whose functions are

certainly distinct (Manen et al., 1993). The sequenced region comprises also the first 56 *rbc*L codons.

The total aligned DNA matrix is available on request from the authors. It is 1152 sites long, including gaps. A careful analysis of this DNA matrix prompted us to discard from the analysis three small regions. These positions are: (1) The position 706–716, which represents the link between the *rbc*L leader sequence and the *atp*B leader sequence. We have discarded from the analysis this highly variable stretch of adenosine nucleotides. (2) The position 875–878, which is subject to an interesting intramolecular recombination of four nucleotides, GTGA, to its complementary TCAC. The mechanism of this recombination is not known, but a stem-loop structure (favored by the surrounding conserved inverted repeat) is certainly involved. These nucleotide changes are obviously not independent and were treated as a unique event. (3) Following the positions 1138–1140, a CG rich region is susceptible to sequencing errors. We have removed these three sites from our analysis.

Phylogenetic (parsimony) analyses of this DNA matrix were conducted with the PAUP program (version 3.1, Swofford, 1991) on a Quadra 700 Macintosh computer. Only phylogenetically informative characters have been analyzed. Except when indicated in the text, heuristic searches have been conducted with 10 replications of random addition of sequences, TBR branch swapping, and MULPARS options. Because of the size of the matrix, only 100 bootstrap replications have been conducted.

Results

We have found two rather large characteristic deletions: the first one specific to the subfamily Rubioideae (position 496–699), and the second one specific to the tribe Rubieae (position 221–294). The former consists of a large (204 nucleotides long) deletion in the *atp*B leader sequence, the latter is an additional smaller (about 50 nucleotides long) deletion that is not found in the other members of the Rubioideae. Therefore, a quick look at the DNA matrix is sufficient to distinguish Rubieae from other Rubioideae, and Rubioideae from other Rubiaceae.

Table 2 represents a record of the variable and informative sites found in the DNA matrix of all the studied taxa, or of the Rubieae alone. To evaluate the influence of indels, gaps treated as new states (the fifth base), or gaps treated as missing data, are also compared. This shows the large effect

Table 2. Record of variable and informative sites. Amount of variable and informative sites in the DNA matrix treated with the gap = newstate or the gap = missing option. These values are also calculated for the Rubieae ingroup alone.

		Variable	Informative
Gap = newstate	All taxa	737	520
	Rubieae	179	95
Gap = missing	All taxa	314	206
	Rubieae	144	68

of gaps in the analysis, as well as the smaller amount of variability within the Rubieae as compared to the other Rubiaceae studied here.

In order to evaluate the influence of the gaps, two different analyses of the DNA matrix were conducted: one with the option "gap = newstate" (gaps treated as multiple additive independent events), and the other with the option "gap = missing" (Fig. 1). (1) For the "gap = newstate" matrix, we used a heuristic search, with 10 replications of random addition of sequences and the TBR branch swapping option. Only three most parsimonious trees were obtained. Figure 1A shows the strict consensus of these trees. They are 1027 steps long, and the consistency index (C.I.), excluding uninformative sites, is 0.659, which is a rather high value. (2) For the "gap = missing" matrix, we used a heuristic search, without replication. Figure 1B shows the consensus tree of the huge amount (more than 1500) of different most parsimonious trees found (392 steps long, C.I. = 0.643).

The consensus tree obtained with the "gap = missing" option is very similar to the consensus tree obtained with the "gap = newstate" option. In spite of minor and non-contradictory dissimilarities, the general topology is conserved. This is encouraging in view of the two very different matrices used, and strongly supports the topology of the phylogenetic tree obtained.

Figure 2 shows a cladogram of one of the three most parsimonious trees obtained with the "gap = newstate" matrix. We chose to present it because it presents the same topology as the consensus tree of Figure 1, while the two others have little more information not sustained by the consensus. The bootstrap values are indicated on the cladogram of Figure 2.

For more details inside the Rubieae, an analysis including only *Theligonum*, *Putoria*, and all the Rubieae species is presented in Figure 3 as a phylogram (in which the branch lengths are propor-

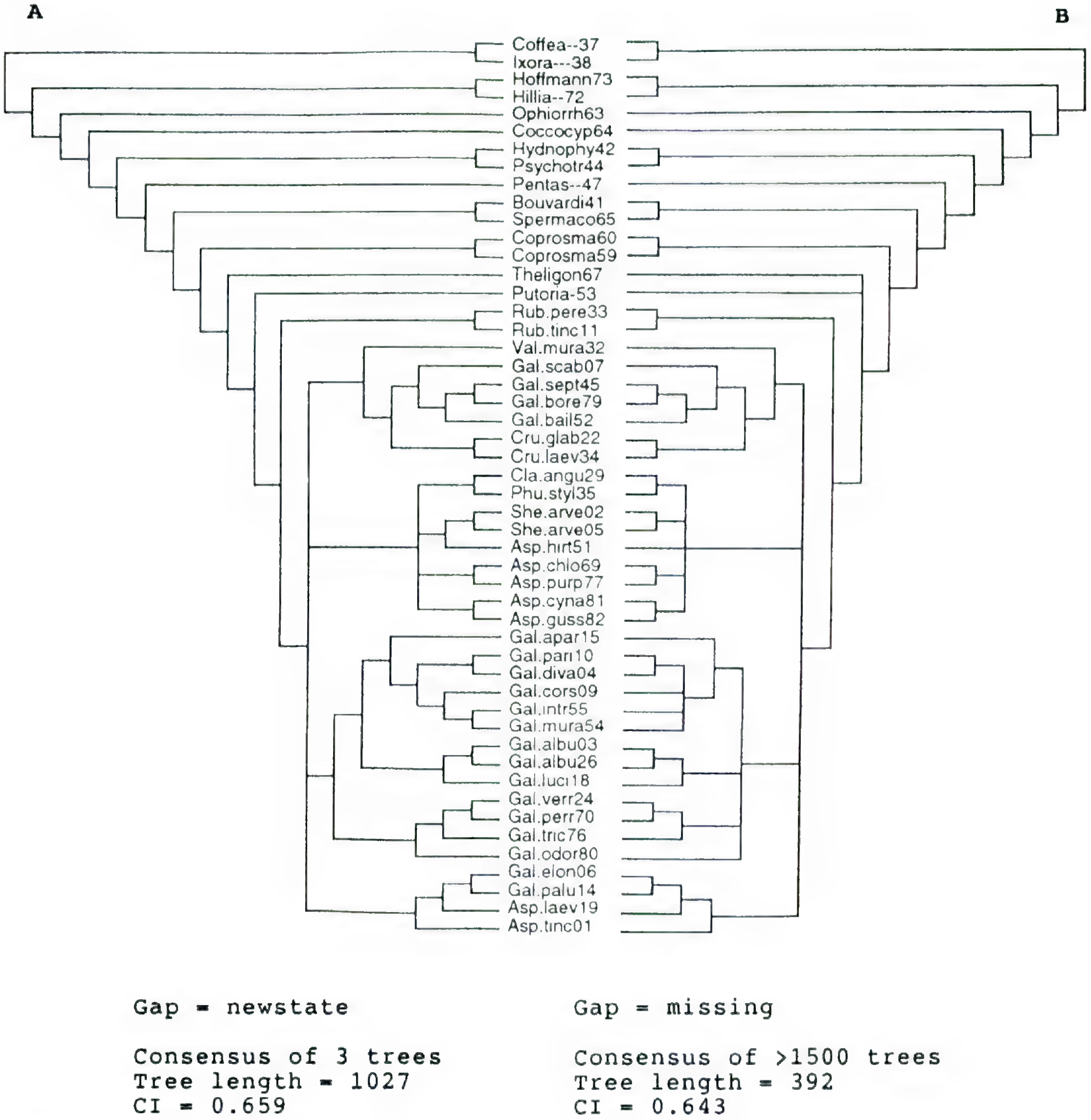

Figure 1. Comparison of the cladograms obtained either with the gap = newstate or the gap = missing option.—A. Strict consensus tree obtained with the option gap = newstate. Three most parsimonious trees were produced.—B. Strict consensus tree obtained with the option gap = missing. More than 1500 most parsimonious trees were produced. For the Rubieae ingroup the species names are indicated by the three first letters of the genus and the first four letters of the species, except for *Crucianella* = Cla. For the other species, the first eight letters of the corresponding genus are indicated. The numbers following the species names correspond to those indicated in Table 1.

tional to the number of steps). We used a heuristic search (gap = newstates option), with 10 replications of random addition of sequences and the TBR branch swapping option. Only three most parsimonious trees were obtained (232 steps, C.I. = 0.694). As explained above, we chose to present the tree as having the same topology as the consensus.

Discussion

As detailed above, we found a strongly supported separation between a first group of taxa (*Coffea, Ixora, Hoffmannia, Hillia, Ophiorrhiza*) from different subfamilies and a second group with the remaining taxa all clearly belonging to the subfamily Rubioideae. These groups are distinguished by 192 steps, which are mostly due to the 204-nu-

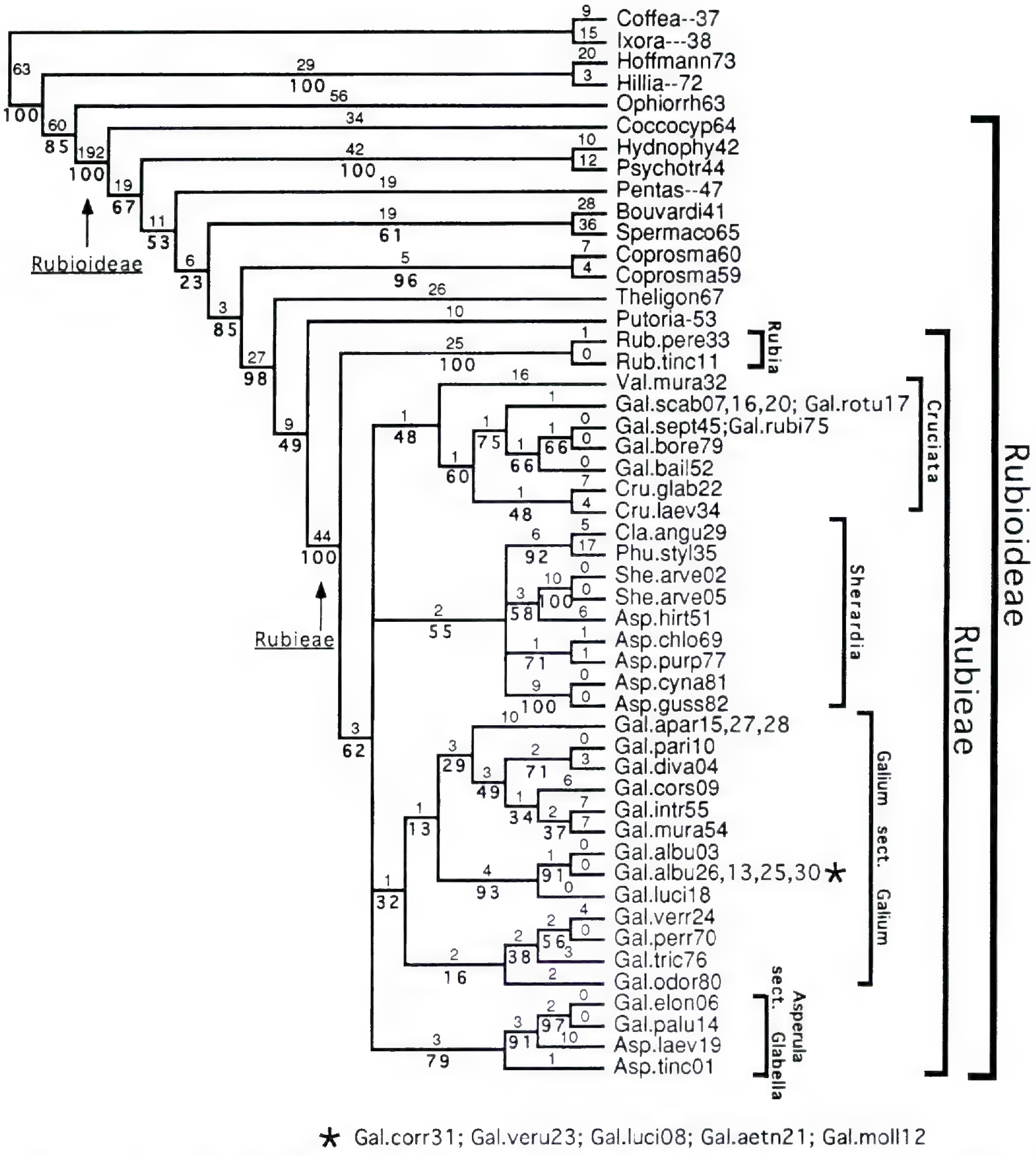

Figure 2. One of the three most parsimonious trees obtained with the option gap = newstate. It presents the same topology as the strict consensus tree of Figure 1A (1027 steps, C.I. = 0.659). The branch lengths are indicated above the branches, and the corresponding bootstrap values of 100 replications are indicated in boldface below the branches. The large deletion 496–699 is specific for the Rubioideae. The deletion 221–294 is specific for the Rubieae. Species coded as in Figure 1. In this figure, species or samples having identical sequences are indicated on the same branch or by the asterisk.

cleotide deletion characterizing the subfamily Rubioideae (Fig. 2).

Within the first group three clades can be recognized: (a) *Coffea* and *Ixora* (treated as outgroup), (b) *Hoffmannia* and *Hillia/Cosmibuena*, (c) *Ophiorrhiza* (Fig. 2). Our parsimony analysis has been conducted using *Coffea* and *Ixora* as outgroups. This choice was determined by the fact that these taxa belong to the subfamily Ixoroideae, which is distantly related to the subfamily Rubioideae analyzed in this study (see also Ehrendorfer et al., 1994).

Hoffmannia, *Hillia/Cosmibuena*, and *Ophiorrhiza* appear strongly separated from the remaining typical Rubioideae taxa by the lack of their characteristic deletion. In view of the still insufficient sampling of Cinchonoideae for our study, we do not want to draw detailed conclusions about the

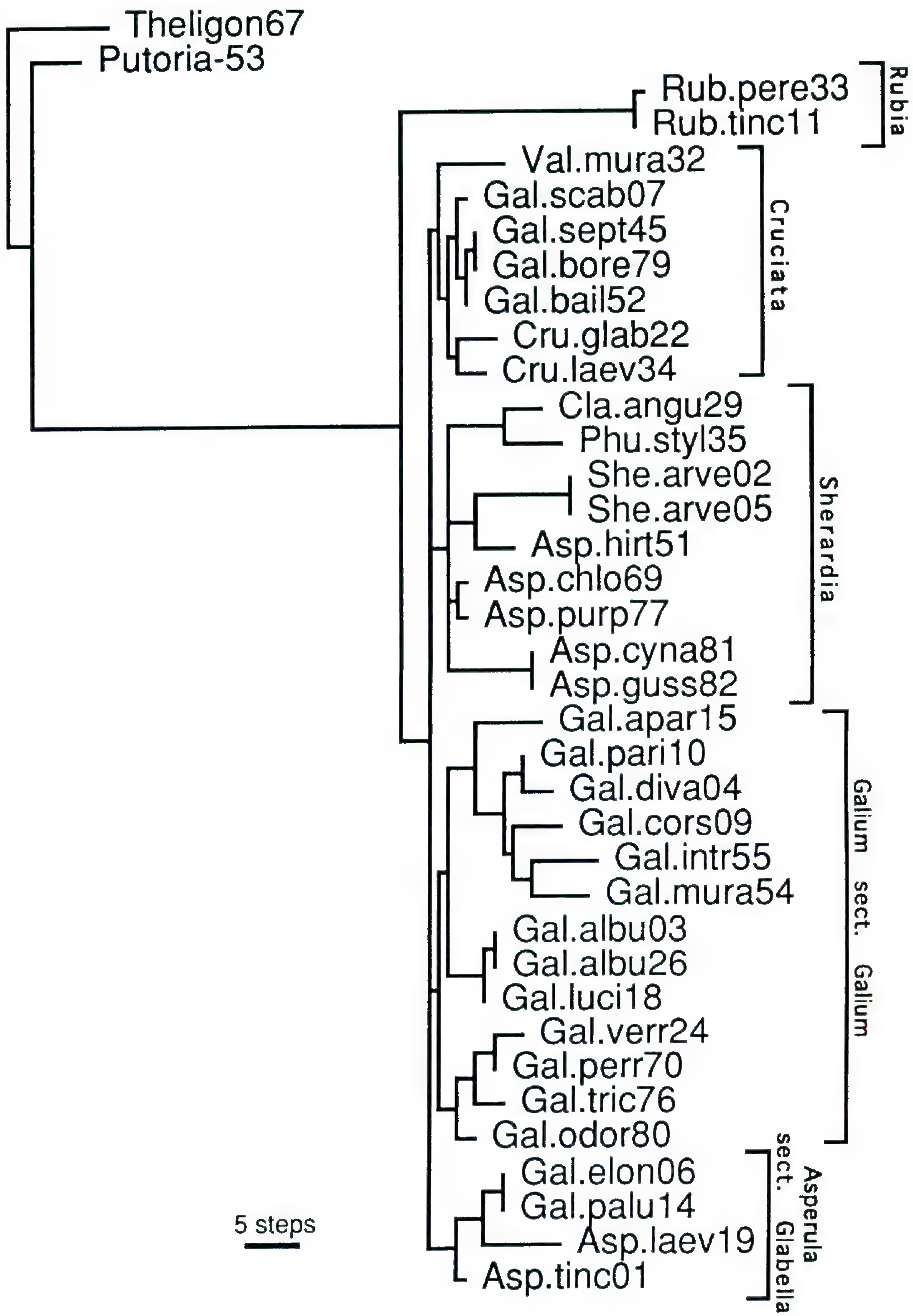

Figure 3. Phylogram (PAUP) including only *Theligonum*, *Putoria*, and the Rubieae species. One of the three most parsimonious trees obtained with the option gap = newstate (232 steps, C.I. = 0.694). Species coded as in Figure 1. For clarity, species or samples having identical sequences are not indicated, and can be followed from Figure 2.

systematics of these taxa. Nevertheless, while our placement of *Hillia valerii* (= *Cosmibuena valerii*) outside the Rubioideae corroborates the most recent works on the tribe Hillieae (Robbrecht, 1988; Andersson & Persson, 1991; Andersson, 1993), the position of *Hoffmannia* and *Ophiorrhiza* is not in accordance with the data of most of the authors. In fact, *Hoffmannia* (represented here by *H. refulgens* from Mexico) belongs to the tribe Hamelieae, which has been included in the subfamily Rubioideae because of the occurrence of raphides (Bremekamp, 1966; Verdcourt, 1958; Robbrecht, 1988). However, the position of Hamelieae quite distant from the remaining members of Rubioideae is in accordance with restriction cpDNA data (Bremer & Jansen, 1991). In a more recent contribution, Bremer & Struwe (1992) affirmed that both molecular and morphological analyses support a position of the tribe Hamelieae outside the Rubioideae. The systematic position of *Ophiorrhiza* has always been very controversial. The views of Verdcourt (1958), Darwin (1976), and Robbrecht (1988), which included this genus in the subfamily Rubioideae close to the Hedyotideae, seem to be contradicted by our molecular data, because *Ophiorrhiza* appears clearly separated from the remaining Rubioideae taxa (including the Hedyotideae genera *Bouvardia* and *Pentas*) by the lack of their characteristic deletion.

The second group of taxa includes unquestionable members of the Rubioideae that share the large 204-nucleotide deletion in the *rbc*L-*atp*B intergene region. This drastic evolutionary change characterizes an obvious monophyly. Our study clearly supports the more modern concept (Robbrecht, 1988) that the presence or absence of raphides should not be overemphasized, as in the system of Verdcourt (1958). Following our molecular data, Rubioideae apparently always have raphides, but there are taxa (*Hillia/Cosmibuena*, *Ophiorrhiza*, *Hoffmannia*) outside this clearly cpDNA-defined subfamily where this character is also present. However, our results are only partially in agreement with the hypothesis presented by Robbrecht (1993) that Rubioideae should essentially be restricted to tribes with uniovulate locules and that the multiovulate species placed in the Rubioideae show more affinity to Cinchonoideae. In fact, our molecular data demonstrate that *Coccocypselum* and some genera of Hedyotideae with multiovulate locules are clearly members of Rubioideae. The independent molecular data from Bremer et al. (1995, this issue) corroborate this view. Our cladogram suggests that the derivation of uniovulate locules occurred several times in parallel from a multiovulate basal phylogenetic line within Rubioideae. This conclusion is in line with Robbrecht's (1993) view that most of the characters used to distinguish major groups in the family evidently have evolved in a parallel fashion several times.

In the following paragraphs we will discuss the clades separated by our molecular analysis within unquestionable Rubioideae (see Fig. 2). Obviously, the fact that only 10 of the 16 tribes that Robbrecht (1988) included in Rubioideae have been analyzed in our study makes our conclusions still preliminary.

Coccocypselum is generally recognized to form a monotypic tribe, Coccocypseleae, placed in the subfamily Rubioideae (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988). This is supported by our data as well as by the results of cpDNA restriction site mapping (Bremer & Jansen, 1991; Bremer & Struwe, 1992). Nevertheless, we cannot confirm the view of these authors of placing *Coccocypselum* between the Psychotrieae and the Hedyotideae clades. According to our data, it constitutes a basal tribe within Rubioideae with multiovulate locules and thick-walled exotestal cells. This is now supported by the most recent work of Bremer et al. (1995, this issue) using *rbc*L sequences.

The Psychotrieae, with consistently uniovulate locules, evidently constitute a clade of the Rubioideae that separated early, as is clearly shown by our intergene sequences as well as by the restriction and *rbc*L data (Bremer & Jansen, 1991; Bremer et al., 1995). The Hedyotideae, clearly belonging to Rubioideae, are another tribe with multiovulate locules, but mostly advanced parenchyma-like exotesta. Whereas *Pentas* was always placed here, *Bouvardia* has been classified in the tribe Cinchoneae (subfamily Cinchonoideae) by Schumann (1891) and, tentatively, by Robbrecht (1988), in contrast to Verdcourt (1958) and Bremekamp (1966), who transferred it because of its raphides to Hedyotideae; the same placement has been suggested by the cladistic analysis of Anderssson & Persson (1991). While all available cpDNA data clearly support this latter view, they raise the problem of a para- or even polyphyletic nature of the Hedyotideae. Whereas earlier suggestions about closer affinities of *Pentas* with Anthospermeae than with *Bouvardia* (Bremer & Jansen, 1991) have been dropped recently (Bremer et al., 1995), our and Bremer's molecular data clearly converge in suggesting that Spermacoceae share ancestry with Hedyotideae. Our cladogram (Fig. 2) shows *Bouvardia* much closer to *Spermacoce* than to *Pentas*,

and Bremer et al. (1995) demonstrate the same from independent data for the Spermacoceae genus *Richardia*. Close relationships between Hedyotideae and Spermacoceae are also suggested by karyological data (Kiehn, 1986, 1995 this issue) and from studies on the genus *Otiophora* (Igersheim & Rohrhofer, 1993). All this supports the idea that the Spermacoceae, with their one-seeded fruit locules, are derived from multi-seeded Hedyotideae-like ancestors.

Our cpDNA intergene data (see Fig. 2) suggest that the remaining tribes of Rubioideae studied here (i.e., Anthospermeae, Theligoneae, Paederieae, and Rubieae) represent a monophylum, in which the character of uniovulate ovary locules has become fixed. The most recent information based on restriction and *rbc*L analyses (Bremer et al., 1995) indicate at least a close proximity of these tribes.

The tribe Anthospermeae, represented here by two species of *Coprosma*, forms a well-separated clade as already suggested by Bremer & Jansen (1991) and Bremer et al. (1995). The monophyly of the Anthospermeae is also supported by several morphological characters (Puff, 1986).

The small tribe Theligoneae, represented here by the Mediterranean *Theligonum cynocrambe*, clearly belongs to the subfamily Rubioideae and appears relatively close to the Paederieae and Rubieae (bootstrap value 98%). The Theligonaceae have been formerly associated with Haloragaceae, Hippuridaceae, and Portulacaceae; only recently, Wunderlich (1971) has suggested an affinity with the Rubiaceae. Our molecular data, together with the recent independent findings of Bremer et al. (1995), now give definite evidence that *Theligonum* belongs to the Rubiaceae/Rubioideae.

Putoria calabrica, the only taxon of the tribe Paederieae analyzed, is the closest to the Rubieae among the taxa considered in our study. This is not surprising, as this Mediterranean genus exhibits obvious morphological affinities (flowers in fascicles, corolla infundibuliform) with several more plesiomorphic taxa of Rubieae.

Finally, the tribe Rubieae is clearly monophyletic according to the available molecular data from 8 genera (out of the 13 to be included in the tribe) and 39 species. This is strongly supported by 44 steps, which are mostly due to a specific 50-nucleotide deletion that does not exist in the remaining Rubioideae (Fig. 3).

The sequence analysis of 14 additional species belonging to the Rubieae has not greatly changed the topology of the tribal cladogram presented previously and based on 25 taxa (Manen et al., 1994). In fact, again five clades can actually be distinguished within the tribe. In our previous work (Manen et al., 1994) these five clades were well separated by all the different methods used for the phylogenetic analysis of the available DNA data.

(1) *RUBIA* CLADE

Our phylogenetic analysis clearly shows the genus *Rubia* as a sister group of the other Rubieae taxa. The separation from the other clades is clear whatever method or matrix is used (Manen et al., 1994), and is supported by a 62% bootstrap value.

In our phylogenetic reconstruction *Rubia* appears to be derived from the ancestor of the Rubieae, while classical treatments based on morphological data usually have considered this genus to be advanced. Nevertheless, the occurrence of plesiomorphic characters in all or part of *Rubia*, e.g., woody growth forms, leaves and leaflike stipules in whorls of four, 5-lobed and sometimes even funnel-shaped corollas, and fleshy drupaceous fruits, suggests a basal position in the tribe.

(2) *SHERARDIA* CLADE

This clade comprises *Sherardia arvensis*, the group of *Crucianella* (abbreviated in the cladogram as Cla; Cru is *Cruciata*) and *Phuopsis*, and the majority of the species studied from the heterogeneous genus *Asperula*. It is not surprising to find *A. cynanchica* and *A. gussonei* from section *Cynanchicae* closely associated. Sequence data support the placement of *A. purpurea* (with rotate flowers and formerly classified as *Galium purpureum*) and *A. chlorantha* (with a long corolla tube) as related in section *Thliphtisa* (Ehrendorfer & Krendl, 1976). *Asperula hirta* (sect. *Hexaphylla*) exhibits some affinities with *Sherardia*, which in turn is also allied to the closely related genera *Crucianella* and *Phuopsis*. Even if the sequence links between all these different subgroups of the clade are not very strong, their inclusion in a monophylum is not surprising in view of several morphological similarities and suspected relationships (see, e.g., Richard, 1829). In our previous publication (Manen et al., 1994) we were surprised to find *Sherardia* far from the *Asperula* species analyzed; but, as we expected, a broader sampling of the heterogeneous genus *Asperula* now has closed the intergene cpDNA gap between it and *Sherardia*.

(3) *ASPERULA* SECT. *GLABELLA* CLADE

The inclusion of several additional species from the genera *Galium* and *Asperula* in our molecular analysis has absolutely not changed the topology

of this clade, which represents one of the most interesting results presented in our previous publication (Manen et al., 1994). On first sight, it was surprising to find that the closely related species *Galium palustre* and *G. elongatum* exhibit much stronger cpDNA affinities with *Asperula* sect. *Glabella* than with the other *Galium* species studied. These two perennial species of swamp and marsh habitats belong to the small section *Aparinoides* (Ehrendorfer & Krendl, 1976). This section is characterized by several aberrant features, such as plants usually turning blackish when dry, obtuse (and not acute) leaves, slightly campanulate flowers, globose (and not ovoid) mericarps, and the chromosome base numbers $x = 12$ (and not 11 as in nearly all other members of *Galium*). All this has made the systematic position of section *Aparinoides* uncertain, and has prompted one of us (F. E.) to suspect an affinity with *Asperula* sect. *Glabella*, where the differential characters mentioned reappear. This suspicion is remarkably supported by the present molecular data and should lead us to reconsider the taxonomic position of section *Aparinoides*, surely separated from the other *Galium* species.

(4) *CRUCIATA* CLADE

The coherence of *Valantia* and *Cruciata* (abbreviated as Cru in the cladogram) in respect to our sequence data is not surprising, since strong morphological and karyological relationships between these two small genera from the Near East, the Mediterranean, and temperate western Eurasia have already been demonstrated (Ehrendorfer, 1971).

We find in the same clade *Cruciata* and *Valantia* together with members of *Galium* sect. *Platygalium*, whereas the latter appears well separated from the taxa arranged inside the *Galium* clade. This justifies our concept of a distinct *Cruciata* clade (Manen et al., 1994), now broadened by the analysis of additional species. This clade is also supported by some important morphological characters, such as pseudowhorls of only two leaves and two leaflike stipules, each often with three parallel veins. The terminal inflorescence (thyrsus) and the hermaphrodite flowers in (most species of) *Galium* sect. *Platygalium* are plesiomorphic features, whereas *Cruciata* and *Valantia* are more advanced with respect to their apically vegetative inflorescence (with lateral cymes) and their andromonoecious flowers.

Galium sect. *Platygalium* comprises a large and diverse group of species with worldwide distribution that is still difficult to define. The sequence analyses of the species studied so far reflect some of its subgroups: *G. scabrum* and *G. rotundifolium* are members of a Western Eurasian/African $2x$-aggregate, whereas *G. baillonii* ($2x$, Carpathian Mts.), *G. boreale* and *G. septentrionale* (polyploids, Eurasia and North America, probably conspecific), and *G. rubioides* (high polyploid, Eurasia) are members of a polymorphic Northern Hemisphere polyploid complex.

The molecular data show that *Valantia* is the sister group to a clade containing *Cruciata* and *Galium* sect. *Platygalium*. This is in contrast with the morphological views (Ehrendorfer, 1971; Ehrendorfer & Krendl, 1976), which suggest that *Valantia* and *Cruciata* are the most closely related taxa and are more advanced than *Galium* sect. *Platygalium*.

(5) *GALIUM* SECT. *GALIUM* CLADE

Monophyly is suggested by our molecular data for this clade, represented here by the perennial sections *Galium*, *Leiogalium*, *Leptogalium*, *Hylaea*, and the annual *Kolgyda*. It is well separated from *Galium* sect. *Platygalium* and section *Aparinoides*. However, its internal relationships are still insufficiently understood. The core of the *Galium* clade, with the two closely related sections *Leiogalium* (with *G. mollugo*, *G. album*, *G. corrudifolium*, *G. lucidum*, *G. aetnicum*) and *Galium* (with *G. verum*), exhibits practically no sequence differences. This is not surprising in view of extensive hybridization documented within and between these sections (e.g., *G. verum* × *G. album*).

Since Linnaeus's (1753) *Species Plantarum* the taxon *Galium odoratum* (sect. *Hylaea*) has been regarded as a core species of the genus *Asperula*, differentiated originally only on the basis of its infundibuliform corolla shape. Nevertheless, there are several typical *Galium* species from Asia with rotate corollas that closely correspond in all other characters with "*Asperula*" *odorata*, e.g., *G. asperuloides*, *G. hofmeisteri*, *G. japonicum*. This, and the lack of morphological links with other, unquestionable members of *Asperula*, has prompted the taxonomic transfer of this and other taxa wrongly placed in *Asperula* to *Galium* (Ehrendorfer, 1948: 232 f.; 1958: 353; Ehrendorfer & Krendl, 1976). This transfer is now convincingly backed by our molecular data.

The grouping inside this clade is not sufficiently supported by the bootstrap and has to be regarded as provisional. *Galium odoratum* (sect. *Hylaea*) and *G. perralderii* (taxonomic position uncertain),

both perennials, may be related and clearly occupy a more basal position within the *Galium* clade. *Galium corsicum* belongs to the short-lived perennials of section *Leptogalium*. All the remaining species studied so far are annuals and have been placed in section *Kolgyda*. Morphologically, however, they form rather distinct subgroups: *G. tricornutum* and *G. verrucosum*; *G. aparine*; *G. parisiense* and *G. divaricatum*; *G. intricatum* and (morphologically highly apomorphic) *G. murale*. Available sequences indicate complex interrelationships and suggest a polyphyletic origin of the section *Kolgyda*.

Conclusions

The results of our molecular study give clear evidence for the monophyletic origin of the tribe Rubieae, at least according to the data from the 8 genera and 39 species that have been analyzed. The Rubieae could have originated from a (sub)tropical ancestor in common with Paederieae in the course of adaptation to temperate regions. Future research could establish the precise geographical location and the age of this ancestor. Further interesting questions will concern the ecophysiological adaptation underlying its change from tropical to temperate environments, and its worldwide migration routes. Similar trends have occurred repeatedly in angiosperm evolution, and are therefore of general relevance.

Within the tribe Rubieae, the 39 species studied so far constitute 5 groups, confirming our previous results (Manen et al., 1994). The obtained data are often in agreement with classical taxonomic views; moreover, in some cases the molecular data strongly support some suspected affinities among systematically distant taxa.

The disparities between the traditional morphological data and our molecular data could be caused by procedural problems, such as equating overall similarity with phylogenetic relationships (Sytsma, 1990). Such discrepancies may also be caused by biological phenomena frequent in Rubieae: unequal rates of morphological evolution can obscure phylogenetic relationships and, moreover, hybridization and polyploidy result in reticulate evolution not resolved by chloroplast sequences.

Our phylogenetic information on the tribes outside Rubieae is obviously still preliminary. However, the discovery of the important deletion apparently specific for the subfamily Rubioideae opens some unexpected opportunities for a rapid and effective delimitation and phylogenetic reconstruction of this subfamily.

This large deletion affects one of the two *atp*B promoters. The deletion of the distal *atp*B promoter in the Rubioideae is a unique feature in the large range of the dicots so far tested (Manen et al., 1993). It would be interesting to look at the biological (metabolic) significance of its sudden disappearance. Since the two *atp*B promoters are suspected to have a specific biological function in the regulation of the expression of the ATP synthase (coupling factor, a key component of the photosynthetic apparatus), the suppression of one of them might have some physiological implications. Could this be linked to the capacity of this subfamily to produce several herbaceous lines, often adapted as colonizers and expanding into xeric and temperate environments?

The results of our phylogenetic study of the Rubieae/Rubioideae, based on sequence data for a chloroplast intergene region, show the great taxonomic potential of this new method. This cpDNA non-coding region shows large amounts of variability from the intrageneric level and upward, offering an effective tool for a phylogenetic approach to the delimitation and relationships of genera, tribes and subfamilies. Nevertheless, these molecular studies will obviously have to be combined with more traditional biosystematic approaches and other molecular methods, since only multidisciplinary cooperation will result in real progress concerning our systematic and phylogenetic understanding of this enormous family.

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A SYSTEMATIC REVISION OF *ADANSONIA* (BOMBACACEAE)[1]

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ABSTRACT

The baobabs (Bombacaceae: *Adansonia*) are tropical trees native to Africa, Australia, and Madagascar but dispersed widely by humans. The members of the genus are united by several derived characters that serve to distinguish them from other Bombacaceae, including a characteristic, indehiscent fruit with reniform seeds and a powdery pulp. The systematics of *Adansonia* is revised, with three sections and eight species being recognized. The support for each species is discussed in the context of the "Genealogical Species Concept." Several nomenclatural problems are resolved, and a new combination, *A. gibbosa* (A. Cunn.) Guymer ex D. Baum, is made. In addition, the ecology, ethnobotany, and conservation status of *Adansonia* is summarized, focusing especially on the poorly known Malagasy and Australian species.

The earliest references to the African baobab, *Adansonia digitata* L., are descriptions of the tree and its water-storage capacity in the travelogue of the 14th-century Arab traveler Ibn Batuta (e.g., 1922, vol. 4: 381; 1929: 322). Occasional mentions of the tree can also be found in the reports of European explorers who visited Africa from the 15th century onwards. For example, Aloysius Cadamosto (1507 in 1937: 70) described a tree of huge girth that was almost certainly a baobab. Likewise, André Thevet (1558 in 1878: 47) described an edible fruit from Cape Verde with kidney-shaped seeds. However, these sources were overlooked and European botanists came to know of *A. digitata* through fruits carried by traders to Egypt. Alpino (1592) described these fruits and their reputed medicinal properties using the name *Bahobab*, probably derived from the Arabic "bu hibab," meaning "many-seeded fruit" (Nicolas, 1955; Wickens, 1983). The description and accompanying illustrations published by Alpino (1592) constituted the main information about the plant for over 150 years (e.g., Clusius, 1605; Veslingius, 1638; Ray, 1693; Lippi, 1704).

In the mid 18th century Michel Adanson visited Senegal and provided the first detailed botanical description and illustrations of *A. digitata* (Adanson, 1757, 1771). He realized the connection to the fruit described by Alpino (1592) and named the plant *Baobab*. Although Linnaeus (1753, 1759) did not retain this name for the genus, preferring instead an honorific for Adanson, the name baobab has persisted as the common name for *A. digitata* and other species of *Adansonia*.

In the early 18th century Allan Cunningham, the naturalist on the HMS Mermaid under Captain King, discovered the "Gouty-stem Tree" in northwestern Australia. He believed it to be a *Capparis* (*C. gibbosa* A. Cunn.), but mentioned that it had a superficial resemblance to the baobabs of Africa (Cunningham, 1827; Heward, 1842). Later voyages to the area (Grey, 1841; Stokes, 1846) added further information, but it was left to Ferdinand von Mueller on the Gregory Expedition to the Victoria River basin to recognize formally its taxonomic affinity with the African baobab and to describe it in the genus *Adansonia* as *Adansonia gregorii* (Mueller, 1857, 1858).

The first record of a baobab in Madagascar appears to be the plates in de Bry's *Indiae Orientalis Icones Quartoe*, published in 1605 (plates 62 and 63 in Teil, 1979). These plates illustrate cu-

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rious, swollen, bottle-shaped trees that clearly correspond to *Adansonia rubrostipa* Jum. & H. Perrier (Keraudren, 1963). Nonetheless, it was not until the late 19th century that the presence of *Adansonia* in Madagascar was recognized (Baillon, 1876). In the late 19th and early 20th centuries several Malagasy species were described (Baillon, 1889, 1890b; Jumelle & Perrier de la Bâthie, 1909a, 1910), but these were based on poor specimens and inaccurate field observations and resulted in much nomenclatural confusion (see, e.g., Hochreutiner, 1908; Jumelle & Perrier de la Bâthie, 1910). Perrier de la Bâthie (1952a, 1955) resolved many of these problems, but a few have persisted until the present.

Apart from *Adansonia digitata*, the genus is poorly known. Within the last 30 years the only scientific work on the Malagasy and Australian species (apart from descriptions in floras) has been a single article by Keraudren (1963), a series of papers by Miège and coworkers (Miège & Burdet, 1968; Cretton, 1972; Miège & Lambert, 1973; Miège, 1974, 1975), and four papers by Armstrong (1977a, b, 1982, 1983). These papers were based largely upon previous taxonomic literature and incorporated few new field observations.

I spent over 14 months in Madagascar and Australia working on the genus *Adansonia*. In the course of this fieldwork, it became clear that significant inaccuracies have been perpetuated in the literature and that a taxonomic revision of the genus was needed. Elsewhere I have documented the pollination and floral biology of all the species (Baum, 1995), and reported new chromosome counts for all members of the genus (Baum & Oginuma, 1994).

Generic Affinities

Adansonia was assigned to Malvaceae by de Jussieu (1789) when he named that family. It was one of the ten genera segregated by Kunth (1822) as the family Bombacaceae, and has remained there ever since. Within Bombacaceae, *Adansonia* is usually united with *Bombax* L. and its allies in the tribe Adansonieae (Schumann, 1890; Bakhuizen van der Brink, 1924; Hutchinson, 1967) or Bombaceae (Edlin, 1935). The tribe comprises *Adansonia*, *Bombax* L. and segregates (Robyns, 1963; Alverson, 1994), and, according to some authors (e.g., Bakhuizen van der Brink, 1924), *Ceiba* Miller and segregates (Gibbs et al., 1988). Members of Bombaceae are usually trees, often with swollen trunks, having palmately compound (rarely unifoliolate), stipulate leaves, 3-bracteolate pedicels, and a distinct androphore (staminal tube). These characters all occur elsewhere in the family, hence the monophyletic status of the tribe is uncertain.

Adansonia occupies an isolated position in tribe Bombaceae (Hutchinson, 1967) and is almost certainly monophyletic. The fruit is unique in the family, consisting of a woody, indehiscent pericarp surrounding numerous, reniform seeds embedded in a chalky or spongy matrix. Similarly, *Adansonia* is unique in the family in that the calyx encloses the floral buds completely, in contrast to the truncate calyx occurring in other genera.

Vegetative Morphology

Baobabs are long-lived (Swart, 1963), small to large trees with broad, sometimes bottle-shaped trunks and relatively compact crowns. Juvenile plants usually have slender, tapering trunks that are often swollen at ground level. Trunks comprise soft, fibrous wood arranged in concentric layers. The wood appears to store water, with a tree's diameter fluctuating depending on rainfall (Guy, 1970, 1971). Surrounding the wood is a thick sheath of longitudinal fibers, and outside that a thin photosynthetic layer beneath a skin of outer bark.

The vegetative and reproductive shoots of baobabs are, to varying degrees, dimorphic. The vegetative axes are usually relatively slender, often green and with long internodes, whereas reproductive axes tend to be stouter, generally woody, and with short internodes. Vegetative and reproductive shoots may also differ in indumentum, petiole length, leaflet shape, and the presence or absence of marginal teeth. However, the degree of shoot dimorphism in the different species and the particular differences between vegetative and reproductive shoots is not sufficiently well characterized to be of systematic use.

Baobab leaves have caducous stipules (persistent in *Adansonia perrieri* Capuron) and are palmately compound. However, seedlings and in some cases regenerating shoots, may have simple (not unifoliolate) leaves. The number of simple leaves produced prior to the switch to compound leaves varies and is a useful character for identifying seedlings. In particular, *A. digitata* (and to some extent *A. madagascariensis* Baill.) seedlings produce simple leaves much longer than the other species. The transition to compound leaves can be gradual, with a variety of intermediate morphologies produced. Leaflets may be entire or toothed and may be glabrous or have simple or clumped trichomes.

Germination is hypogeal, but there is great variation in the length of the hypocotyl. In section

Brevitubae the hypocotyl is very short, with the cotyledons remaining at soil level, usually still encased in the remains of the testa ("cryptocotylar"). In other species, the hypocotyl is longer (though there is much variation between species), and the cotyledons usually break free of the testa ("phanerocotylar").

Reproductive Morphology

Inflorescences of baobabs are borne in the axils of leaves close to the tips of reproductive branches. The inflorescence almost always comprises a single flower whose flower stalk is made up of a proximal peduncle and a distal pedicel demarcated by a distinct joint. This implies reduction from an ancestral multiple-flowered inflorescence, which gains support from the occasional occurrence of pairs of flowers having distinct pedicels but a shared peduncle.

The flowers are large, showy, and odoriferous and in all cases open just before or soon after dusk. Anthesis occurs rapidly in all species, generally taking less than 30 minutes. Some species of section *Longitubae* have flowers that open in as little as 30 seconds (Baum, 1995). The movements of the flower parts are sufficiently rapid to be detected with the naked eye. Although in some species flowers may remain attached to trees for several days, all the pollen is released during the first night and the stigmas become shriveled on the first morning. Thus the flowers are in their reproductive phase for at most 15 hours.

The calyx in *Adansonia* spp. is usually green (brown in *A. grandidieri* Baill.). The lobes are valvate and fused almost to their tips in bud. At anthesis the five lobes tear apart and become either reflexed or coiled at the base of the flower. The separation of the lobes is not precise, and frequently two or more adjacent lobes remain fused to each other. When adjacent lobes are fused they reflex, but usually less completely than single lobes, and often distort the shape of the flower.

The unsplit portion of the calyx, the "tube," varies in shape from flat to cup-shaped to tubular and is one of the key characters for separating the sections. The calyx has a ring of nectar-producing tissue near its base, which may be localized in an annular swelling of the calyx tube in some members of section *Longitubae*.

A useful systematic character is calyx persistence. In section *Longitubae* (except *Adansonia gibbosa* (A. Cunn.) Guymer ex D. Baum) the calyx drops with the other flower parts, whereas in *A. gibbosa*, section *Brevitubae*, and section *Adansonia* the calyx is persistent.

The petals are inserted on the staminal tube a few millimeters above its base. The variation in the shape of the petals provides useful diagnostic characters. Petal length is useful for distinguishing *Adansonia rubrostipa*, with petals significantly shorter than the androecium, from *A. za* Baill. and *A. madagascariensis*, with which it can otherwise be confused. Petal color varies from white, through cream to bright yellow and dark red. However, due to the fact that most collections of *Adansonia* are of fallen rather than fresh flowers, petal color has proved to be a source of confusion. Even flowers that are pure white at anthesis appear cream or yellow after they have sat on the ground for a few days. Furthermore, once flowers have completely dried out they turn a reddish brown, which may have led Perrier de la Bâthie (1952a, 1953, 1955) to the false conclusion that *A. suarezensis* H. Perrier has red rather than white petals.

The androecium comprises a sturdy staminal tube below, with numerous free filaments above. The length of both the tube and the free filaments varies greatly and is important for delimiting the sections and species. In addition, the fusion of a central bundle of filaments above the top of the staminal tube is a diagnostic character in *Adansonia rubrostipa* (though this is usually not easy to see on pressed flowers).

The gynoecium consists of a densely pubescent ovary enclosed in the staminal tube, a long style emerging from the middle of the free filaments, and a terminal stigma. In several species, especially *Adansonia digitata*, the style becomes bent over or kinked in the floral bud. However, this may be a developmental phenomenon and it is not a fixed character in any one species. In contrast, stylar abscission is useful in identifying fallen flowers. Whereas most species have styles that persist after floral abscission, *A. madagascariensis*, *A. rubrostipa*, and *A. perrieri* have styles that fall as a unit with the androecium and other flower parts.

The fruit of *Adansonia* is best described as a large, dry berry with an indehiscent woody pericarp, a spongy or chalky "pulp," and numerous reniform seeds. The fruit of *A. gibbosa* is unique in the genus in that it is functionally dehiscent, tending to crack irregularly while still on the tree and usually breaking open upon falling to the ground. Fruit shape and pericarp thickness vary among species but are also highly variable within *A. digitata*, *A. gibbosa*, and *A. za*. These and other characters of fruit morphology (e.g., pericarp anatomy) seem to have great potential in the systematics of *Adansonia*, but have yet to be fully exploited.

For much of its range, *Adansonia za* Baill. is

characterized by fruit with a markedly swollen peduncle. This character is, however, absent from some northern populations of *A. za*, and thus, though useful for identification, is not diagnostic. Likewise, *A. za* fruits often, but not always, have longitudinal ridges which serve to distinguish it from other members of the genus.

Seeds, though always more or less reniform, vary in size and shape. The two species of section *Brevitubae* are distinguished from the others by having larger seeds that have a relatively shallow sinus and are not laterally flattened. Among the other species there is variation in seed size and sinus depth, but much is intraspecific and thus seed characters are difficult to use for identification or diagnosis at the species level. In the species descriptions seed dimensions are given in a standard format: length × width (dorso-ventral) × width (lateral), where the seed's ventral surface is defined as the sinus.

Anatomy and Palynology

While the anatomy of the African species, *Adansonia digitata*, is fairly well known (e.g., Gerber, 1895; Braun, 1900; Metcalfe & Chalk, 1950; Rao, 1952, 1954; Van Heel, 1966, 1974; Inamdar & Chohan, 1969; Davis & Ghosh, 1976), little work has been carried out on the Malagasy or Australian species (but see Gerber, 1895). Clearly, further comparative work is needed.

The palynological work has also been restricted primarily to *Adansonia digitata* (Fuchs, 1967; Sharma, 1969; Vaishampayan & Sharma, 1981), but data are available for the Australian species, *A. gibbosa*, and the Malagasy species *A. grandidieri*, *A. rubrostipa* and *A. madagascariensis* (Presting et al., 1983; Nilsson & Robyns, 1986). The pollen grains (ca. 60 μm diam.) are 3-porate/pororate, suboblate to prolate spheroidal. The pores are 3–8 μm, rounded to oval, and the exine is microreticulate to tectate-perforate and is adorned with blunt processes (ca. 0.5–2.0 μm). The studies to date have not suggested any palynological characters that have any use in infrageneric classification.

Phytogeography

Adansonia is almost exclusively tropical, extending only a few degrees south of the tropic of Capricorn in Madagascar and Africa (cultivated populations of *A. digitata* occur in the northern subtropics, e.g., Florida, U.S.A.). Section *Brevitubae* (two spp.) is restricted to Madagascar; section *Longitubae* (five spp.) has one species in northwestern Australia and four in Madagascar; and section *Adansonia* comprises one species that is native to continental Africa but has been widely dispersed by humans.

The African/Australian disjunction was formerly explained as being the result of continental drift (e.g., Maheshawari, 1971; Raven & Axelrod, 1972; Aubréville, 1975). With improvements in our knowledge of the history of the southern continents (e.g., Rabinowitz et al., 1983), this scenario now seems improbable. Instead, it is now thought that *Adansonia* arrived in Australia via long-distance dispersal (Raven & Axelrod, 1974; Armstrong, 1983). Circumstantial support comes from the occurrence of water dispersal in the genus (see below) and the arrival of drifting fruits of *A. digitata* on Aldabra (Wickens, 1983: but note the reference given, Wickens (1979), makes no mention of fruit arriving on Aldabra). Dispersal to Australia probably occurred within the last 30 million years, after Australia entered the tropics.

Pollination Biology

Full details of the pollination biology of the Malagasy and Australian species of *Adansonia* are given in Baum (1995), but a brief summary follows.

The African baobab, *A. digitata*, has long been known to be bat-pollinated (e.g., van der Pijl, 1934; Porsch, 1935; Jaeger, 1945, 1950, 1954; Start, 1972), though other visitors, including bushbabies, do visit the flowers and may occasionally bring about pollination (Coe & Isaac, 1965; Wickens, 1983). By extrapolation, it was argued that the remaining baobabs would also be bat-pollinated (van der Pijl, 1956; J. Armstrong, 1979), although P. Armstrong (1977a, 1983) suspected bird-pollination. My work in both Madagascar and Australia found that the situation is more complex.

Both species in section *Brevitubae* receive visits from moths, bees, and sunbirds but are pollinated only by mammals. I have observed fruit bats visiting and pollinating *A. suarezensis*, whereas the only pollinatory visits to *A. grandidieri* were those of the nocturnal lemur *Phaner furcifer*. Further observations would be useful, however, to rule out bat-pollination of the latter species and to determine if any nocturnal lemurs visit the former.

All species of section *Longitubae* studied to date are pollinated by long-tongued hawkmoths. The Australian species, *Adansonia gibbosa*, is visited by the hawkmoth *Agrius convolvuli*. *Adansonia perrieri*, *A. za*, and *A. rubrostipa* are pollinated by *Coelonia solanii*, with additional pollination by

Xanthopan morgani (*A. perrieri*), *Coelonia brevis* (*A. za*), and *Coelonia mauritii* (*A. za*). Nocturnal lemurs in Madagascar and honeyeaters in Australia were important floral visitors that may contribute somewhat to pollination. In contrast, other animals visiting *Longitubae* flowers (bees, flies, ants, settling moths, and sunbirds) seemed to function exclusively as nectar or pollen thieves.

Dispersal Biology

The pulp of baobab fruit is tasty and nutritious, being a particularly rich source of potassium tartrate, tartaric acid, and vitamin C (Greene, 1932; French, 1944; Carr, 1955; Nicol, 1957; Nour et al., 1980). The seeds, while having a high lipid content (de Wildeman, 1903; Rey, 1912), are surrounded by a very tough, thick testa.

In Africa the main dispersal agents of *Adansonia digitata* are terrestrial mammals, particularly elephants and baboons (Wickens, 1983). Enhanced germination rates have been reported for seeds that have passed through an animal's digestive system (Baker, in Rick & Bowman, 1961), suggesting that the African baobab is primarily endozoochorous.

It has recently been determined that the Australian baobab, *Adansonia gibbosa*, is also dispersed by terrestrial mammals, in this case kangaroos and wallabies (Done, 1987; Baum & Handasyde, unpublished data). Fallen fruits are eaten and the seeds pass intact. However, unlike *A. digitata*, there was no significant difference in the germination percentage of seeds collected from droppings (2%) versus seeds taken directly from fruit (5%) (Baum & Handasyde, unpublished data). In view of the viability of seeds that have been eaten by mammals and the large number of seeds that are eaten (shown by the frequency of droppings containing seeds), we may conclude that *A. gibbosa* is primarily endozoochorous. However, the suggestion of bat-dispersal in *A. gibbosa* (Froggatt, 1934) seems unlikely.

In Madagascar, animal dispersal of *Adansonia* has not been documented. In view of the intensive ecological and behavioral work conducted on lemurs, the only extant animals potentially capable of acting as seed dispersers, it appears that the baobabs of Madagascar are not currently endozoochorous. However, among the many animals that have become extinct since humans colonized Madagascar (ca. 1500–2000 years ago; Mack, 1986) are some that could have been seed-dispersers of the Malagasy baobabs. The most likely candidates for this role are two species of the subfossil genus *Archaeolemur* (L. Godfrey, pers. comm.). They were abundant throughout much of Madagascar until human colonization and persisted until at least 1000 years ago (D. Burney, pers. comm.). They are thought to have been similar in size, habits, and locomotion to baboons (*Papio* spp.) and were "primarily frugivorous, or at least dietarily generalized, consuming large food items, such as hard-skinned fruits" (Tattersall, 1982: 220). Another candidate worth considering is the elephant bird, *Aepyornis* spp. (P. Armstrong, pers. comm.), a large terrestrial ratite (the heaviest bird that ever lived) that occurred primarily in the south and west of the country as recently as the 17th century. They were potentially important seed-dispersers, as their "very powerful beak would enable [them] to dig up tubers and open large fruit" (Mahé, 1972: 344).

In the absence of animals as seed-dispersers, it seems that water offers the only means of dispersal available to the extant Malagasy baobabs. The buoyant fruit and tough waterproof pericarp of *Adansonia digitata* means that dispersal along rivers, on floodwaters, and along strandlines is probably frequent (Wickens, 1983). In contrast, water dispersal may be ruled out for the Australian species on account of its fragile, usually dehiscent pericarp. In Madagascar, water dispersal occurs in *A. perrieri* growing alongside rivers. For example, in Montagne d'Ambre numerous fruit may be found in the Rivière de Makis floating as much as 1/2 km below the nearest tree (pers. obs.). Similarly, *A. za* and *A. madagascariensis* fruit, which have tough, indehiscent pericarps and are frequently found along watercourses, are likely to be water dispersed. However, hydrochory is unlikely in *A. grandidieri* and *A. suarezensis* because of their fragile pericarps and in *A. rubrostipa* because it rarely occurs near rivers or streams.

Taxonomic Treatment

Genealogical species concept employed

In view of the current lack of consensus as to how species are to be defined, it is necessary to specify the criteria I have employed in the following taxonomic treatment. In evaluating whether a group of organisms constitutes a species, I have evaluated whether there is evidence that they show "exclusivity of descent," wherein each member of the group is genealogically more closely related to all other members of the group than to any individuals outside the group (de Queiroz & Donoghue, 1990). The concept of genealogical relatedness I have in mind is, however, not the conventional notion of

recency of descent from a common ancestor, but rather the recency of common ancestry of all their genes when considered simultaneously. Under this view of relatedness (and hence exclusivity), species are composed of organisms that constitute a clade on every gene tree (although the arrangement within the clade will vary from gene to gene). The practical and theoretical implications of this perspective on species are discussed in more detail elsewhere (Baum & Shaw, 1995). The important point to note here is that in order to become a species a group must be genetically isolated from other groups for many generations. The actual number of generations varies, depending especially on the population size. The recently developed branch of population genetics called *coalescent theory* (e.g., Hudson, 1990; Maddison, 1995) shows that smaller populations (or those that have passed through bottlenecks) take less time to become exclusive than larger ones.

Because the criterion of exclusivity applies not just to species but also to higher taxa, species are defined as "basal" exclusive groups, i.e., groups that contain within them no nested, exclusive groups (Baum & Shaw, 1995). In this treatment of *Adansonia,* I have hypothesized that groups of organisms are species when they share a number of fixed differences from their nearest relatives and contain no subgroups that could be exclusive. This follows because groups that have diagnostic characters (especially if they are apomorphic characters), are likely to have been isolated from other such groups, and thus have a high probability of being exclusive. Nonetheless it should be stressed that, unlike the "Phylogenetic Species Concept" (Cracraft 1983, 1989; Nixon & Wheeler, 1990; Davis & Nixon, 1992), the possession of diagnostic characters is *evidence* of species status but does not in itself *confer* species status. Ultimately, the species hypothesized in this paper could be tested using, for example, genealogical analysis of molecular variation (discussed in Baum & Shaw, 1995; Baum & Donoghue, in press). In the meantime, however, the species proposed here should be seen as the best estimates of the genealogical units existing within *Adansonia.*

In the case of *Adansonia,* I believe that the genealogical species I have described are coextensive (i.e., have similar boundaries) with the species that would be recognized by applying other widely held species concepts, for example: the "Biological Species Concept" (Mayr, 1942), the "Phylogenetic Species Concept" (Cracraft, 1983; Nixon & Wheeler, 1990), the "Cohesion Species Concept" (Templeton, 1989), or the traditional phenetic approach. Thus, even if my perspective on the definition of "species" is rejected, the taxonomy I propose may be seen as having some general validity.

METHODS

This treatment is based upon herbarium studies and fieldwork conducted during a total of a year in Madagascar (four visits) and two and a half months in Australia (one visit).

The measurements given in the species descriptions below are based primarily on fresh material. Depending on the way they were dried and whether collections comprise freshly picked material or fallen and already desiccated flowers, flowers may have shrunk by as much as 50%. Well-pressed specimens have measurements that approximately match those made on fresh material.

Adansonia L., Syst. Nat. ed. 10, 2: 1144. 1759. *Baobab* Adans. Fam. ii 399. 1763. *Ophelus* Lour., Fl. Cochin. 412. 1790. *Baobabus* Kuntze, Rev. Gen. 1: 66. 1891. TYPE: *Adansonia digitata* L.

Deciduous trees 5–30 m tall, with unarmed trunks and usually compact crowns. Trunks massive, cylindrical, tapering from bottom to top or bottle-shaped. Primary branches massive, unarmed (rarely with conical spines in *A. rubrostipa*), horizontal or ascending. *Bark* smooth or foliaceous, reddish brown to gray, often with a yellow or green photosynthetic layer close to the surface. Inner bark layers with tough, longitudinal fibers. Wood fibrous and soft, arranged in concentric sheets with an odorless, mucilaginous gum often exuding from damaged wood. *Leaves* palmately compound, membranaceous to coriaceous, arranged spirally with very short internodes at the tips of reproductive branches, but with longer internodes on juvenile shoots; deciduous during dry season. Stipules up to 2 mm long, caducous, rarely 1.0–1.5 cm and persistent. Petiole bipulvinate. Leaflets 5–11, elliptic, lanceolate or oblanceolate, lateral leaflets smaller than medial, sessile or petiolulate; apex acute (rarely obtuse) to apiculate; margins entire or toothed; lamina glabrous to tomentose with simple or clumped hairs. *Flowers* perfect and hypogynous, in single-flowered, axillary inflorescences (very rarely 2 flowers to an inflorescence), up to 5 per branch apex. Peduncle and pedicel ± distinct, the latter with usually 3 caducous bracteoles. Buds subglobose, ovoid or cylindrical. *Calyx* synsepalous, enclosing flower until at least 6 hours before anthesis; calyx lobes 5 (but lobes may fail

to separate completely), reflexed or contorted at base of flower; scabrous and green or brown outside; sericeous and cream, pink, or red within; nectariferous tissue in a narrow strip at the base, sometimes within an annular cavity formed by the base of the calyx tube. *Corolla* actinomorphic or slightly zygomorphic; petals 5, free, convolute, inserted on the lowermost portion of the staminal tube, creamy white, yellow, or red. *Androecium* comprising a distinct tube surmounted by numerous free filaments; tube cylindrical or slightly tapering, usually glabrous, white or yellowish; anthers monothecate with the thecae long and sinuous around the margin of the connective, longitudinally dehiscent. *Ovary* superior, conical to oblong, densely brown or yellowish tomentose, with cylindrical or flattened, long, simple, upward-pointing hairs; ovules numerous, anatropous, borne on long, dichotomizing funicles arising from the edges of 7–9 deeply intruded placentae. Style simple, filiform, densely tomentose near base, white or dark red; stigma usually with 5–10 short lobes, yellowish or dark red, browning with cessation of receptivity. *Fruit* a dry, large, many-seeded berry; globose, ovoid, or oblong-cylindrical; pericarp reddish or blackish brown, tomentose, hard and woody (5–15 mm thick) with longitudinal fibers on the inner surface; usually indehiscent. Seeds enveloped in a white or cream, dry, powdery or spongy pulp. Seeds 10–15(–20) mm long, reniform; cotyledons 2 (rarely polycotylous), ± equal, folded and achlorophyllous. Seedlings cryptocotylar or phanerocotylar. Juvenile leaves initially simple, later lobed, trifoliate or irregularly digitate.

Nomenclature. Although the type species, *A. digitata*, appears in *Species Plantarum* (Linnaeus, 1753), the generic name was omitted from *Genera Plantarum*. Hence, the correct date of publication is 1759, when Linnaeus validly published the generic name in *Systema Naturae* (Jarvis et al., 1993).

KEY TO THE SECTIONS AND SPECIES OF *ADANSONIA*

1a. Flower buds ovoid to oblong (approximately twice as long as broad), on short, ± erect pedicels; staminal tubes ca. 1 cm long, approximately one-fifth as long as free filaments; seeds 12–20 mm long and not laterally flattened; trees with flat-topped crowns; Madagascar. .... I. sect. *Brevitubae*
- 2a. Leaflets (6–)9–11, bluish green, densely tomentose, narrowly elliptic to lanceolate; fruit subglobose to ovoid, usually less than 2 times as long as wide; outer surface of calyx reddish brown; restricted distribution in southwestern Madagascar. .... 1. *A. grandidieri*
- 2b. Leaflets 6–9, yellowish green, subglabrous to scabrous, broadly elliptic to broadly oblanceolate; fruit elongated ovoid to oblong-cylindrical, usually more than 2 times as long as wide; outer surface of calyx green; restricted distribution in northern Madagascar. .... 2. *A. suarezensis*

1b. Flower buds very elongated (at least 5 times as long as broad) or globose (equally long and broad), pedicels erect, horizontal or pendent; staminal tubes 3–20 cm, at least one-third the length of the free filaments; seeds 10–14 mm long, laterally flattened; trees with usually rounded crowns; tropics.
- 3a. Flowers and fruit borne on long, pendulous flower stalks; buds globose; petals white, as broad as long; semiarid continental Africa; cultivated and naturalized in other tropical regions. .... II. sect. *Adansonia*; 3. *Adansonia digitata*
- 3b. Flowers and fruit borne on short, erect or horizontal flower stalks; buds elongated-cylindrical; petals white, red or yellow, at least 5 times as long as broad; Madagascar and Australia. .. III. sect. *Longitubae*
  - 4a. Flower buds usually 10–15 cm; petals white or cream, erect for lower ¾ of length, reflexed above; fruit usually cracked when mature; rocky outcrops, creek beds and floodplains in northwestern Australia. .... 4. *Adansonia gibbosa*
  - 4b. Flower buds usually 15–28 cm; petals yellow or red, curled outward evenly throughout length; fruit indehiscent; Madagascar only.
    - 5a. Leaflets with serrate margins, sessile, medial leaflets less than 2 cm wide; petals shorter than androecium; central 10–20 filaments fused into a bundle above the top of the staminal tube; style caducous with other flower parts; fruit ± globose; deciduous forest on sand or limestone, dry "spiny" forest or sublittoral scrub in western and southern Madagascar. .... 5. *Adansonia rubrostipa*
    - 5b. Leaflets with entire margins, petiolulate or subsessile, medial leaflets greater than 2 cm wide; petals longer than androecium; filaments free from top of staminal tube; style persistent or caducous; fruit spherical, ovoid or oblong.
      - 6a. Staminal tube at least 8 times longer than the free filaments; leaflets obovate to obovate-elliptic, 15–24 pairs of secondary veins on reproductive shoots; stipules persistent; fruit broadly ovoid to oblong, peduncle not swollen; northern Madagascar. .... 8. *Adansonia perrieri*
      - 6b. Staminal tube equaling or shorter than the free filaments; leaflets broadly lanceolate or elliptic with usually 8–20 pairs of secondary veins on reproductive shoots; stipules caducous; fruit oblong or globose, but if oblong then the peduncle is usually markedly swollen.

7a. Leaflets long-petiolulate (up to 3 cm) to sessile, 10–20 pairs of secondary veins; style persists after floral abscission; fruit usually ovoid with a markedly thickened peduncle, or broadly oblong to subglobose with normal pedicel/peduncle; longer than wide; flowering November to February; widespread and variable species from extreme southern to northwestern Madagascar. .... 7. *Adansonia za*

7b. Leaflets subsessile with winged petiolules, 8–16 pairs of secondary veins; style caducous; fruit globose to subglobose, usually wider than long; flowering February to April; northwestern to northern Madagascar. .... 6. *Adansonia madagascariensis*

I. Adansonia section **Brevitubae** Hochreutiner, Ann. Conserv. Jard. Bot. Genève 1907/1908: 136. 1908. TYPE: *Adansonia grandidieri* Baill. (lectotype, here designated).

Large trees with massive cylindrical or tapering trunks and flat-topped crowns. Flowering during dry season (May–August). Flower buds brown or green, ovoid, approximately 2 to 3 times as long as broad; borne on short, erect peduncles/pedicels. Petals white, oblanceolate, approximately 5 times as long as broad. Androecium white; staminal tube ca. 1 cm × 1.5 cm diam., free filaments 600–1100, 3–7 cm long. Fruit oblong, ovoid, or subglobose with persistent calyx bases and a dense, reddish brown indumentum; pericarp thin and fragile. Seeds large (12–20 mm long), reniform, not flattened (i.e., lateral width approximately equal to dorso-ventral width). Germination cryptocotylar.

Nomenclature. In his description of this section, Hochreutiner (1908) listed two species: *Adansonia grandidieri* and *A. za*. In the latter case, Hochreutiner was referring to *A. za* sensu Drake (= *A. suarezensis* Perr.) rather than *A. za* Baill. Thus, to avoid possible confusion, *A. grandidieri* is selected as lectotype.

1. Adansonia grandidieri Baillon, Hist. Nat. Pl. Plates 79Bbis/2, 79E/1. 1893. TYPE: Madagascar. Morondava, *Grevé 275* (lectotype, here designated, P).

Large deciduous trees up to 25 m tall and 3 m diam., with usually single, cylindrical trunks (tapering from bottom to top when young) and flat-topped crowns; primary branches regularly distributed, mainly horizontal. *Bark* reddish gray and smooth. *Leaves* (6–)9–11-foliolate; petiole 5–13 cm, 1–3 mm diam., pubescent; stipules caducous; leaflets shortly petiolulate (1–5 mm), medial leaflets 6–12 × 1.3–3 cm, lanceolate to narrowly elliptic, bluish green when fresh; densely tomentose with short, clumped, yellowish hairs; margins entire. *Flowers* emerging when leafless; buds erect, ovoid; flower stalk ca. 1 cm diam., dark brown tomentose; peduncle 3–5 mm long; pedicel 6–8 mm long. *Calyx* lobes (3–)5, reflexed and twisted at the base of the flower, 7.5–8.5 cm long, 1.5–2 cm wide; reddish brown tomentose outside, cream villous within. Calyx tube forming an open cup ca. 1 cm deep within. *Petals* white (yellowing with age), twisted, narrowly lanceolate or oblanceolate, approximately 5 times as long as broad, ca. 9–10 cm × 17–20 mm. *Androecium* white, comprising a staminal tube, ca. 8–10 × 14–16 mm, surmounted by 600–700 free, spreading filaments, 3.5–6.5 cm long. *Ovary* broadly rounded-conical, 1.1 cm high, with dense, upward-pointing, lemon-yellow hairs; containing ca. 300 ovules. *Style* white, straight or rarely slightly kinked; usually 2–3 cm longer than the central filaments, densely villous below, glabrous above, fitting loosely into the staminal tube and persisting after floral abscission. Stigma white or pinkish, with very short irregular lobes. *Fruit* subglobose to broadly ovoid with persistent calyx bases and a dense reddish brown indumentum; pericarp 2.5–4 mm thick, fragile, with few longitudinal fibers. Seeds reniform, not markedly laterally flattened, 12–14 × 10–12 × 9–10 mm. Germination cryptocotylar.

Specimens examined. MADAGASCAR. **Toliara Province:** Marofandelia, ca. 40 km NW of Morondava on road to Belo-sur-Tsiribihina, 44°33′E, 20°08′S, alt. 20 m, 20 July 1989 (fl), *Baum 321* (MO, TAN); Morondava, center of the town by the hotel Menabe, 44°16′40″E, 20°17′30″S, alt. 3 m, 25 Dec. 1991 (l), *Baum 345* (MO, P, TAN); Morombe, May 1963 (fl), *Chabonis s.n.* (TAN); Taolampia, Morondava (l, fr), *Grevé 20* (BM, G, K, P); Morondava, 1891 (fl, fr), *Grevé 275* (P); Morondava (fl), *Grevé s.n. (a)* (P); Taolampia (Morondava), Apr. 1894 (fl), *Grevé s.n. (b)* (P); Marosalaza forest, 50 km N of Morondava, alt. 0 m, 13 Dec. 1973 (fl), *Hladik 27* (P); near Morondava (l, fl, fr), *Keraudren-Aymonin & Keraudren 25860* (P); Morondava (fr), *Keraudren-Aymonin & Keraudren 25918* (P); Marofandelia, Morondava, Nov. 1914 (l), *Perrier de la Bâthie 4805* (P); Morondava, May 1910 (l, fl), *Perrier de la Bâthie 8172* (P); N of Tulear, Lac Ihotry near village of Andreketa, 21°55′S, 43°37′E, alt. 50 m, 1 Jan. 1989 (l), *P. B. Phillipson 3063* (MO, P); District of Morondava, Canton of Analaiva, Marofototra, 44°24′E, 20°17′S, 8 Jan. 1954 (l), *SF (Collector #76) 45* (P, TEF). **Without Further Locality:** Sep. 1956 (l), *J. Bosser 9907* (TAN), 1895 (fr), *Grandidier s.n.* (P), 1897 (fr), *Grandidier s.n.* (P), Without date (fr), *Grevé F6771* (P).

Distribution (Fig. 1). Southwestern Madagascar between Lac Ihotry (near Morombe) and Ber-

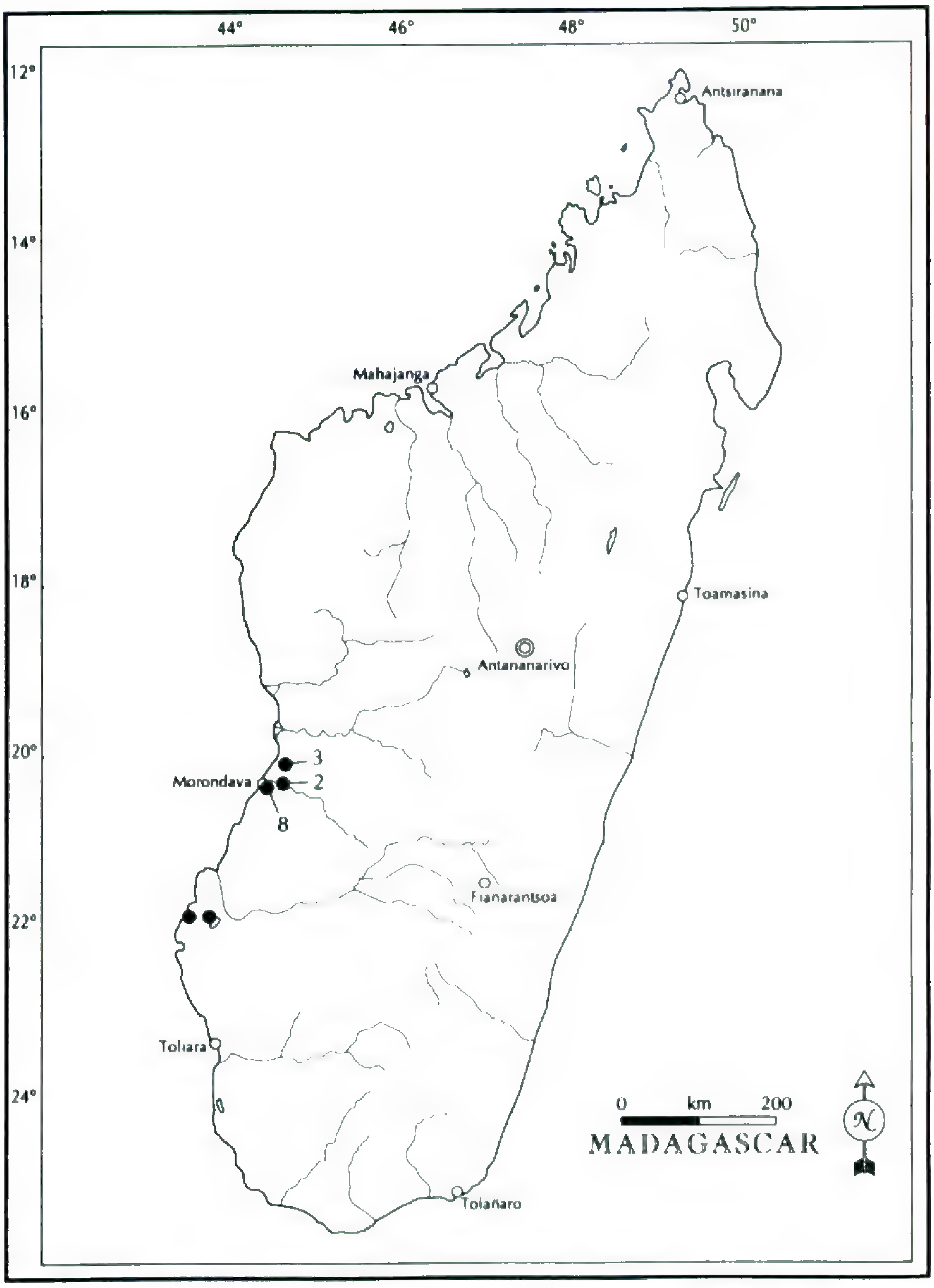

Figure 1. The distribution of *Adansonia grandidieri* in Madagascar. Collection sites are marked with a circle. When more than one collection is made from the same locality the number of separate collections is indicated.

eboka (ca. 50 km north of Morondava). Dry deciduous forest, especially close to seasonal rivers, lakes, and waterholes. Now mainly found in degraded agricultural lands.

Phenology. In leaf from October to May. Flowering May to August. Fruit ripe in November and December.

Cytology. Miège (1974) reported a chromosome count of $2n = 60$–64. Mangenot & Mangenot (1962) and Baum & Oginuma (1994) found $2n = 88$.

Taxonomy and nomenclature. *Adansonia grandidieri* is diagnosed by a combination of characters (see the key) including at least two that are unique in the genus and probably autapomorphic: bluish green and densely stellate-pubescent leaves, and a dark brown floral bud. It is geographically and phenetically isolated from its nearest relative (*A. suarezensis*), manifests no significant internal variation, and is, thus, recognized as a species.

According to Article 44.1 of the *Code* (Greuter et al., 1988), a name of a species is validly published before 1908 by "an illustration with analysis showing essential characters." As noted by Cheek (1989), many taxa of Malagasy plants were published as analytical drawings in the *Histoire Naturelle des Plantes* that accompanied the *Histoire Physique, Naturelle et Politique de Madagascar* edited by Alfred (and later Guillaume) Grandidier. Baillon was reponsible for most of the botanical names associated with the plates, including those illustrating species of *Adansonia* (Stafleu & Cowan, 1976: 979–981). Thus, the correct author of

A. grandidieri is Baillon, and the date of publication is 1893, when plates 79B*bis*/2 and 79E/1 were issued.

The question of typification is problematic. Cheek (1989) argued that there is less ambiguity if the plate itself is treated as the holotype rather than attaching the name to one of the specimens from which the plate was drawn. However, a specimen has the advantage that it has not been "filtered" through an artist's eyes and, thus, future systematists have the ability to score the type for characters absent from the illustration (e.g., trichomes, stomata, DNA). Additionally, in typifying an illustration there is some ambiguity as to whether specific elements of plates, whole plates or sets of plates issued simultaneously should be typified. Thus, the decision must be made on a case by case basis. If a good quality specimen used by the author is available it should be typified. Failing that, it is preferable to typify the plate rather than select a poor quality specimen or a neotype.

The main element of plate 79E/1 is a longitudinal section of a flower showing several important features of *Adansonia grandidieri*. This illustration was, I believe, based on *Grevé 275*, as this specimen includes a sectioned flower that closely resembles the drawing. However, although *Grevé 275* may be designated the type, it cannot be considered the holotype because it is not the only specimen that contributed to the species description. Specifically, the fruit illustrated on plate 79B*bis*/1 resembles that of *Grevé 20*. Thus, I designate *Grevé 275* as lectotype of *A. grandidieri*.

Ethnobotany. *Adansonia grandidieri* is known locally as "renala" or "reniala" (meaning "mother of the forest") and is the most widely exploited of all the Malagasy baobabs (Baillon, 1890a; Perrier de la Bâthie, 1952b). The fruit pulp and seeds are eaten fresh, and cooking oil is extracted from the seeds. The fruit are collected from the ground or with the aid of wooden pegs that are hammered into the trunks and used to climb the trees. In some villages near Morondava, the fruits are fed to goats, who digest the pulp but pass the seeds intact. These are then collected and used for oil-extraction (T. Willing, pers. comm.). The oil extracted from *A. grandidieri* seeds is of good quality, and economic exploitation has been considered (Rey, 1912). Perrier de la Bâthie (1952b) reported that from 1874 until the early 20th century, the seeds of *A. grandidieri* were exported to Marseilles for the extraction of cooking oil. However, according to Perrier de la Bâthie (1952b) the supply of fruit was insufficient to sustain the market.

Keraudren (1963) reported that around the turn of the century the fruit of *Adansonia grandidieri* was exported to England and used to make small, dry tea cakes. However, it is unclear whether Keraudren was referring to the use of the pulp or seeds, and she does not give her historical sources.

In a full-grown tree, the bark is 10–15 cm thick, and composed of tough longitudinal fibers that are used to make ropes, particularly for use on outrigger sailing canoes (Perrier de la Bâthie, 1952b). A majority of *Adansonia grandidieri* show signs of having been used as a source of fiber for ropemaking. The bark is cut off a living tree from ground level to about 2 m. Although the scar persists, bark regenerates over the damaged tissue.

The wood is spongy and moisture-rich, comprising concentric sheets of fiber that probably correspond to annual growth rings. The Malagasy collect these fibrous sheets from either dead or living trees, dry them in the sun, and sell them in local markets for use as thatch. In addition, the undried wood is sometimes fed to cattle in periods of drought.

On account of its spectacular appearance it is not surprising that *Adansonia grandidieri* is the focus of superstitions and folklore. Individual trees are often considered the dwelling places of spirits. Offerings are made at the foot of these trees: grains or other food items for a good harvest, money for fortune, and rum for general prayers and fertility. Giant land snail shells arranged at the foot of a tree (repositories for rum offerings) indicate its sacred status.

Conservation. Most mature *Adansonia grandidieri* now grow in open, agricultural land or degraded scrub rather than in forests. There is a noticeable lack of young trees in these disturbed ecosystems, suggesting that recruitment is low. Of course, it is difficult to be sure that the skewed population structure is caused by disturbance without knowledge of the demography of undisturbed populations. Nonetheless, there are several factors acting in disturbed habitats that might adversely affect recruitment (e.g., fire, seed predation, competition from weeds, and an altered physical environment). Without the ability to perpetuate themselves, the conservational outlook for *A. grandidieri* is far from hopeful. However, Perrier de la Bâthie may have been overly pessimistic when he noted: "Actuellement l'espèce n'est plus représentée que par quelques pieds dont il ne restera rien dans quelques années" (Perrier de la Bâthie, 1952b:

287) [At present this species is only represented by a few individuals of which there will be none remaining within a few years].

2. **Adansonia suarezensis** H. Perrier, Notul. Syst. 14: 300–304. 1952. TYPES: Madagascar. Diégo-Suarez (= Antsiranana), 1893, *Allaud s.n.(a)* (lectotype, here designated, P); *Coridou F6769* (syntype, P).

Large deciduous trees up to 25 m tall and 2 m diam. Trunks single, cylindrical or tapering from bottom to top. Crowns flat-topped; primary branches regularly distributed, mainly horizontal, sometimes with irregular protuberances on their upper surfaces. *Bark* grayish brown and smooth with a yellowish green layer beneath the surface. *Leaves* 6–9-foliolate; petioles 12–18 cm × 2–4 mm, ridged, glabrous; stipules caducous; petiolules 3–5 mm; medial leaflets 11–17 cm × 3.5–5.5 cm, broadly elliptic-oblanceolate, lateral leaflets 6–10 cm × 3.0–3.5 cm; yellowish green, subglabrous to scabrous; margins entire. *Flowers* produced when leafless; buds erect, ovoid; flower stalk ca. 1 cm diam., peduncle 3–5 mm long, pedicel 8–10 mm long. *Calyx* lobes 5, succulent, reflexed and twisted at the base of the flower; green scabrous outside, cream villous within; calyx tube forming a nectar-containing cup ca. 1.5 cm deep. *Petals* white, yellowing with age, oblanceolate, flat or somewhat twisted, approximately 5 times as long as wide, ca. 8–10 × 1.5–2 cm. *Androecium* white comprising a staminal tube ca. 1 cm long × 1.5 cm wide, surmounted by 800–1100 5–7-cm-long, free, spreading filaments. *Ovary* cylindrical to somewhat conical with dense, upward-pointing, golden hairs. *Style* white, straight, densely villous below, glabrous above, fitting loosely into staminal tube and usually persisting after floral abscission. Stigma white, club-shaped, entire. *Fruit* irregularly oblong-cylindrical to elongated ovoid, 20–40 cm long and 8–14 cm wide, with persistent calyx bases and a dense brown indumentum; pericarp 3–4 mm thick, fragile, with few longitudinal fibers. *Seeds* reniform, not markedly laterally flattened, ca. 17–20 × 13–15 × 13–14 mm. Germination cryptocotylar, but sometimes forming an unusual resting stage in which the swollen radicle projects 4–5 cm above the soil while the cotyledons and epicotyl remain at ground level.

Specimens examined. MADAGASCAR. **Antsiranana Province:** Antsiranana District: Diégo-Suarez, 1893 (fl, seeds), *Allaud s.n. (a)* (P); S side of Antsiranana to Ramena road where it passes close to the Montagnes des Français, 49°20′E, 12°19′S, alt. 10 m, 15 June 1989 (fl), *Baum 319A* (MO, P, TAN); Beantely forest on W side of the Antsiranana to Cap d'Ambre road, ca. 10 km N of Antsahampano, 49°10′E, 12°16′S, alt. 100–150 m, 21 June 1989 (fl), *Baum 320A* (MO, P, TAN); Diégo-Suarez, 1896 (l, fr), *Coridou F6769* (P); Diégo-Suarez, 1933 (fl), *Goudot s.n. (b)* (G); Ihotrofrafhy (fr), *Keraudren-Aymonin & Keraudren 25612* (P); near Diégo-Suarez on Basalt, Sep. 1926 (fl, fr), *Perrier de la Bâthie 17686* (G, K, P); Montagnes des Français near Betahitra on Karst, 25 May 1955 (fl), *SF 14225* (P, TEF); limestone mound on road from Diégo-Suarez to Orangea, 8 June 1970 (fl), *SF (Capuron) 29223* (TEF).

Distribution (Fig. 2). *Adansonia suarezensis* is restricted to the very northern tip of Madagascar around the Baie d'Antsiranana. Its precise distribution around the bay is poorly known, but most of the sites known are in deciduous forest, especially on limestone. Whereas small individuals can be found close to the sea in heavily disturbed sublittoral scrub (e.g., 2–5 km south of Cap Diégo or along the Ramena Road, close to the Montagnes des Français), populations of larger trees are found as emergents in less disturbed deciduous forest on steep slopes (e.g., in the southwestern Montagnes des Français, Windsor Castle, and Beantely).

Phenology. Flowering late May to early July. Fruit ripe in November. In leaf, December to April.

Cytology. Miège (1974) reported two conflicting chromosome counts for this species, $2n$ = 48 or 72. Baum & Oginuma (1994) found $2n$ = 88.

Taxonomy and nomenclature. Adansonia suarezensis is diagnosed by a unique combination of characters (see the key), but none of these characters is clearly apomorphic. Nonetheless, *A. suarezensis* is most likely an exclusive group in view of its small population size and presumably long period of genetic isolation, as indicated by its geographic isolation and the fixation of several characters in its probable sister group, *A. grandidieri*. Thus, in the absence of any significant variation within *A. suarezensis*, I consider it a valid species.

In the *Histoire Naturelle des Plantes* (Baillon, 1893: plates 79C, 79I), *Adansonia suarezensis* is illustrated but is incorrectly labeled "*A. za.*" Drake (1902) and Hochreutiner (1908) continued to misapply the name "*A. za*" until corrected by Jumelle & Perrier de la Bâthie (1910). However, Jumelle and Perrier de la Bâthie assumed that the taxon illustrated by Baillon (1893) was *A. grandidieri*, whose flowers closely resemble *A. suarezensis.*

Perrier de la Bâthie (1952a) recognized that the baobabs occurring near Diégo-Suarez (recently ren-

Figure 2. The distribution of *Adansonia suarezensis* in northern Madagascar. Collection sites are marked with a circle. When more than one collection is made from the same locality the number of separate collections is indicated. Some additional localities known by the author are marked with a triangle.

amed Antsiranana) were distinct from the southwestern *Adansonia grandidieri* and described them under the name *A. suarezensis*. However, the descriptions in Perrier de la Bâthie (1952a, 1955) are mistaken in one regard. Probably due to confusion with the sympatric *A. madagascariensis*, or perhaps through observations of dried flowers, the inner calyx and petals are incorrectly described as red.

The type of *Adansonia suarezensis* must be selected from the two specimens listed as "type de l'espèce" by Perrier de la Bâthie (1952a). Perrier de la Bâthie (1955) was therefore incorrect in selecting *Perrier de la Bâthie 17686* as lectotype, because this specimen was labeled "co-type" rather than "type de l'espèce" in the original species description. Of the two syntypes, I have selected *Allaud s.n. (a)* as lectotype because it includes flowers, whereas *Coridou F6769* includes only leaves and fruit.

Ethnobotany. The common name of *Adansonia suarezensis* is "bozy" (pronounced "boojy"), the name used for all the northern Malagasy baobabs. Fruit and seeds are eaten. An infusion of bark is said to cure diabetes. Perrier de la Bâthie

(1953) suggested that *A. suarezensis* seeds, like *A. grandidieri*, might be a valuable source of oil.

Conservation. Although *Adansonia suarezensis* is not quite as rare as suggested by Perrier de la Bâthie (1953), it has a very restricted distribution and is nowhere abundant. The deciduous forests close to Antsiranana are being rapidly devastated, primarily for charcoal and timber. Although the baobabs are usually left standing so they can be exploited for fruit, there is no evidence of any regeneration in these disturbed ecosystems. Already there are very few remnant forest patches left intact and, thus, at the current rate of destruction *A. suarezensis* is likely to be ecologically extinct (i.e., unable to regenerate *in situ*) within the next decade or two. In view of this poor outlook, *A. suarezensis* is certainly an endangered species.

II. **Adansonia** section **Adansonia**

Adansonia section *Pendentes* Hochreutiner, Ann. Conserv. Jard. Bot. Genève 1907/1908: 136. 1908.

Trees with irregular, often very broad trunks and irregular crowns. Flowering during the wet or dry season. Flower buds globose, approximately as long as broad; borne on long pendulous peduncle/pedicels. Petals white, broadly obcordate, equally long and broad. Androecium white; staminal tube ca. 3–6 cm long, free filaments 720–1600, ca. 3–5 cm long. Fruit varying in shape, persistence of the calyx, and indumentum; pericarp thick and woody. Seeds usually less than 12 mm long, reniform, and laterally flattened. Germination phanerocotylar.

3. Adansonia digitata L., Syst. Nat. ed. 10, 2: 1144. 1759. *Adansonia bahobab* L., Sp. Pl. 2: 960. 1763. *Adansonia baobab* Gaertn., Fruct. 2: 253, t. 135. 1791. *Boababus digitata* (L.) Kuntze, Rev. Gen. 1: 66. 1891. TYPE: 862.1 LINN (lectotype, designated by Robyns, 1980).

Ophelus sitularius Lour., Fl. Cochin. 412. 1790. *Adansonia situla* (Lour.) Spreng., Syst. 3: 124. 1826. *Adansonia scutula* Steud., Nom. Bot., ed. 2: 24. 1840. *Adansonia integrifolia* Raf., Sylva Tell., 149. 1838. TYPE: Not seen, probably destroyed.

Adansonia sphaerocarpa A. Chev., Végét. Rég. Tombouctou. In Actes Congrès Int. Bot. 1900: 271. 1901. TYPE: Mali. Timbouctou, Fouta-Djalon, *Chevalier 12424* (holotype, P).

Adansonia digitata var. *congolensis* A. Chev., Bull. Soc. Bot. Fr. 53: 493. 1906. TYPE: San-Thomé Island. Near the town of San-Thomé, *Chevalier s.n.* (holotype, P not located).

Adansonia sulcata A. Chev., Bull. Soc. Bot. Fr. 53: 494. 1906. TYPE: Congo. Brazzaville, *Chevalier 4230* (holotype, P).

Adansonia somaliensis Chiov. Fl. Somala, 8: 30. 1932. Provisional name and therefore invalidly published.

Usually massive deciduous trees reaching 25 m in height and up to 10 m diam., with single or multiple, cylindrical or fluted, often buttressed trunks and spreading, rounded crowns. Branches irregularly distributed, massive. *Bark* gray, smooth to irregularly tuberculate. *Leaves* 5–7(–9)-foliolate; petioles pubescent or glabrous; stipules caducous; leaflets sessile to subsessile, varying greatly in size, medial leaflet 5–15 × 3–7 cm, usually elliptic-obovate, with acuminate apices and decurrent bases; glabrous or with simple or clumped hairs; margins entire. *Flowers* produced during dry or wet season; buds globose with an acute-conical apex, solitary, rarely paired; flower stalk pendulous, 15–90 cm long. *Calyx* lobes (3–)5, triangular, green and tomentose to scabrous outside, cream and villous within, reflexed, 5–9 × 3–5 cm, fused into a broad (5 cm diam.) disc below. *Petals* white, crumpled in bud, broadly obovate, approximately equal in length to breadth, 4–8 × 4–8 cm, narrowing to a thickened base. *Androecium* white, comprising a 3–6-cm cylindrical or tapering staminal tube, surmounted by 720–1600 (Davis & Ghosh, 1976) free filaments, ± equal in length to the staminal tube. *Ovary* conical-ovoid or globose with a thick indumentum of upward-pointing hairs; 7–9 deeply intruded placentae. *Style* white, bent over at right angles or rarely straight; densely villous below, glabrous above, fitting loosely into staminal tube and persisting after floral abscission. Stigma white with irregular lobes. *Fruit* variable; globose to ovoid to oblong-cylindrical, calyx lobes persistent or caducous; pericarp up to 8–10 mm thick, woody, with few embedded longitudinal fibers, covered in a velvety indumentum of yellow-brown or greenish hairs. Seeds reniform, laterally flattened, 10–13 × 8–10 × 4–5 mm. Germination phanerocotylar.

Representative specimens examined. WEST AFRICA. BURKINA FASO: Ouagadougou to Sapone, km 15–16, Bassayam, 12°12′N, 1°33′W, 18 Nov. 1980 (l, fr), *J. Lejoly 80/217* (BR). CAPE VERDE ISLANDS: near Porto Prago, St. Iago, 7 May 1861 (l), *Anon s.n.* (E). CAMEROON: Marona, Aug. 1945 (fl, l), *A. Vaillant 100* (P). DAHOMEY: Mid Dahomey, Bassas country, Bassas–Zoumé and vicinity, 9–10 May 1910 (l, fl), *Chevalier 23641* (P). GHANA: Amansare, 2 July 1913 (fl, l), *N. P. Ashanti 513* (K). GUINEA: banks of the Niger, 19 Jan. 1909 (fl, l), *Chevalier 20453* (K, P). IVORY COAST: Ferkessedougou to Ouango Fitini, 13 Mar. 1969 (l), *P. Bamps 2227* (BR). MALI: Ian (?), around village, 24 June 1899 (l, fl), *A Chevalier 1104* (P). NIGER: without further locality, 1857–1859 (fl, l), *Barter 1528* (GH).

SENEGAL: Kaslack distr., Menirah Village, 5 May 1940 (l, fl), *J.-G. Adam 27199* (MO). TOGO: NE Agouenyivé (Agouévé), 17 Apr. 1978 (fl, l), *Zepernik 124* (K). SIERRA LEONE: Jigaya, 28 Sep. 1914 (l, fl), *N. W. Thomas 2799* (K). CENTRAL AFRICA. ZAIRE: M'vuazi, 28 Dec. 1949 (l, fl), *R. Devred 648* (BR, K). NORTH/EAST AFRICA. CHAD: Bogor Siéké near Logone, 26 Aug. 1969 (l), *Fotius 1689* (P). EGYPT: Cairo, *Schweinfurth 1605* (US). ETHIOPIA: road from Gondar to Axum, 265 km from Gondar near Takazza River, 5 June 1968 (fl, l), *G. J. H. Amshoff 4973* (BR, MO). KENYA: Meru National Park, just S of Rojewero main bridge, 18 Dec. 1972 (l, imm. fr), *J. Ament 444* (K); Mombassa near Lineoni Ferry, Feb. 1970 (fl), *Tweadie 3782* (K). SOMALIA: S Somalia, Bur Akaba, E foot of Mountain, 20 June 1983 (fl, l), *J. B. Gillett & C. F. Hemming 24910* (K). OMAN: Wadi Ghazir, 2 July 1982 (l, buds), *Maconochie 3523* (E, K). SUDAN: Djerme (l), *Chevalier 3055* (BR). TANZANIA: Iringa distr., Ruaha National Park, 2km NNW of Msembe at Mbagi track, 22 Oct. 1970 (fl), *A. Bjornstadt 657* (K). Zanzibar, Massazine, 25 Nov. 1960 (l, fl), *H. G. Faulkner 2735* (BR, K). YEMEN: Taiz Prov., Samsarah, 35 km S of Taiz on road to Turbah, 23 May 1984 (l, fl), *K. J. Gordon 3300* (E). SOUTHERN AFRICA. ANGOLA: near Andongo, Feb. 1857 (l, fl), *Welwitsch 5415* (BM). BOTSWANA: Umtali distr., Hot Springs, 22 Oct. 1948 (fl, l), *N. C. Chase 3728* (BM). MALAWI: Northern prov., Nkhata Bay distr., Nkata Bay, Chikale Beach, 5 Dec. 1976 (fl), *J. Pawek 12000* (K). MOZAMBIQUE: Mulanye distr., Nambwale village near Tunitulu hill, Lake Chilwa, 27 Nov. 1980 (fl, l), *J. D. Chapman 5188* (BR). NAMIBIA: Ombalantu, 1 Apr. 1973 (l, fl, fr), *R. J. Rodin 9201* (ECON, MO). SOUTH AFRICA: Zoutspansberg, Transvaal, 12 Nov. 1932 (fl, l), *Obermeyer et al 69* (K). ZAMBIA: 2 mi. N of Sinazezi, Gwerabe valley, *White 2627* (K). ZIMBABWE: Victoria Falls, 13 Nov. 1919 (fl, l), *L. Shantz 416* (K). INDIAN OCEAN. COMORES: Mayotte, Chissioi Caroni (islet off SW coast), 27 July 1979 (fl, l), *D. Lorence 2800* (MO). MADAGASCAR: Mahajanga, large tree in center of town near the sea, 20 Oct. 1991 (l, fl), *Baum 329* (MO, P, TAN); around villages near Lac Gnamby, near Mt. Tsitondroina, Dec. 1905 (l, fl), *Perrier de la Bâthie 1019bis* (P); around village of Anaboringy near Soalala (Ambongo), Mar. 1903 (l), *Perrier de la Bâthie 5734* (P). FRANCE. RÉUNION: St. Denis, Nov. 1973 (l, fl), *F. Friedman 2461* (P). MAURITIUS: Port Louis, 17 Dec. 1975 (l, fl), *D. Lorence & J. Guého 17639* (P). ASIA. INDIA: Banda, NWP, Aug. 1901 (l, fl), *A. Bell 712* (K); Madras, St. Thomas Mount, 1856 (fl, l), *Drew s.n.* (E). SRI LANKA: Mannar district, Tirukketisvaram, 11 Dec. 1970 (fl, fr), *F. R. Fosberg 53631* (US); Jaffna district, Delft Island, *A. Robyns 6966* (BR, E, K). TAIWAN: Pingtung City, *Chang 10168* (A). PACIFIC. HAWAII. **Oahu:** Honolulu, Queen's Hospital, 14 Sep. 1950 (fl, l), *O. Degener 20716* (NY). CARIBBEAN. ANTIGUA: Donovans Estate, 26 Sep. 1938 (fl, l), *H. E. Box 1550* (BM). GRENADA: St. Georges, July 1905 (l, fl), *W. E. Broadway s.n.* (F). CUBA: vicinity of Soledad, Santa Clara Prov., Aug. 1940 (fl, l), *R. A. Howard 4188* (A). HAITI: Pleine Centrale, Michel de l'Adalaye, 18 Feb. 1925 (l, fl), *E. L. Ekman 3258* (US). MARTINIQUE: without further locality (fl), *P. Duss 2032* (NY). PUERTO RICO: Mita, Mayaguez, 1 Oct. 1981 (l, fl), *A. H. & P. Liogier 32387* (NY). ST. CROIX: Ham's Bay, 16 July 1897 (l, fl), *J. J. Ricksecker 451* (E, F). ST. KITTS: Bassterre, the park, Aug. 1967 (fl, l), *R. K. Wadsworth 579* (A). U.S.A. **Florida:** Fairchild Botanical Garden, 15 May 1970 (l, fl), *W. T. Gillis 9418* (A).

Distribution. The distribution of *Adansonia digitata* was previously described by Miège (1974), Lucas (1971), and Wickens (1983). It is indigenous in semiarid sub-Saharan Africa, extending from Angola, through Southern Africa to East Africa, as far north as southern Sudan and Ethiopia. The extensive populations in West Africa are isolated from those in East Africa by a major, and not fully explained, gap which includes the whole of the Central African Republic (Miège, 1974; Wickens, 1983). It should be noted that the current distribution of *A. digitata* in Africa is partly anthropogenic with naturalized populations, e.g., in Zaire (Miège, 1974; Wickens, 1983). It has been introduced by humans throughout the tropics (see specimen localities above).

Wickens (1983) reported that in Africa *Adansonia digitata* is mainly found in drier lowland areas (up to 1250 m in Sudan) having 200–800 mm annual rainfall (extremes of 90 mm and 1400 mm). Well-drained sandy soils seem to be preferred, although the plants are also found on lateritic soils around the margins of seasonal pools and along rivers.

The populations in Madagascar have been the subject of some controversy. Miège (1974) suggested that the Malagasy *Adansonia digitata* could be relics of the populations that colonized continental Africa. However, since most *A. digitata* in Madagascar are associated with villages, it is more likely that they were recently introduced by Arab traders (Jumelle & Perrier de la Bâthie, 1910; Perrier de la Bâthie, 1952b; Wickens, 1983). This latter explanation is supported by the documented transport of *A. digitata* to Zanzibar, India, and Sri Lanka (see Burton-Page, 1969; Vaid, 1978; Wickens, 1983).

Phenology. Varies greatly between localities. Leaves are shed during the dry season. Flowering can occur in the presence or absence of leaves.

Cytology. Chromosome counts from East and West Africa are $2n = 144$ (Baker & Baker, 1968, Miège & Burdet, 1968) and $2n = 160$ (Baum & Oginuma, 1994), with $2n = 96$ and 128 reported for Southern Africa (Riley, 1960; Schröder in Wickens, 1983).

Taxonomy and nomenclature. *Adansonia digitata* has many diagnostic characters, including several that are unique in the genus and likely to be apomorphic, e.g., a pendulous flower, globose buds, and broad petals. I thus have no doubt that

A. digitata is an exclusive group. However, the question of whether *A. digitata* is a basal, exclusive group is harder to answer. Chevalier (1906) argued for the subdivision of *A. digitata* into at least three species diagnosed by differences in fruit shape. However, I see no evidence that fruit shape diagnoses discrete groups and hence reject Chevalier's taxonomy. Without further studies of variation within *A. digitata* I cannot rule out the existence of exclusive subgroups, but in the absence of evidence *for* such subgroups, (e.g., fixed differences between West Africa, East Africa, and Southern Africa), I advocate that *A. digitata* be treated at the species rank.

Ethnobotany. A substantial body of literature documents the multitudinous uses of the roots, hollow trunks, bark, wood, leaves, flowers, and fruit of *Adansonia digitata*. Reviews and access to the ethnobotanical, anthropological, and economic literature are provided by: Chevalier (1906), Dalziel (1948), Adam (1962), Watt & Breyer-Brandwijk (1962), Owen (1970, 1974), Guy (1971), Wickens (1979, 1983), Armstrong (1983), Burkill (1985), and von Maydell (1986).

Conservation. Despite ongoing ecosystem destruction in Africa, *Adansonia digitata* is conservationally secure because of its wide ecological tolerance. Nonetheless, as pointed out by Wickens (1983), some management may be useful in ensuring that the species survives in areas where it is threatened by tree-clearance or destruction by elephants.

III. Adansonia section **Longitubae** Hochreutiner, Ann. Conserv. Jard. Bot. Genève 1907/1908: 136. 1908. TYPE: *Adansonia madagascariensis* Baill. (lectotype, here designated).

Small to large trees with cylindrical, tapering or bottle-shaped trunks and rounded or irregular crowns. Flowering during the wet season. Flower buds green, elongated-cylindrical, at least 5 times as long as broad; borne on short erect flower stalks. Petals white, yellow, or red; narrowly oblanceolate to narrowly lanceolate, at least 5 times as long as wide. Androecium white or pale yellow; staminal tube 10–25 × 1–1.5 cm; filaments 100–350, 2–12 cm long; usually free, rarely a central bundle of 10–20 filaments fused for about half their length. Fruit globose to ovoid with persistent or caducous calyx bases and a sparse to dense, reddish brown or greenish indumentum; pericarp varying in thickness. Seeds at most 14 mm long, reniform, laterally flattened. Germination phanerocotylar.

Nomenclature. Hochreutiner (1908) described this section and listed three species, *A. madagascariensis*, *A. gregorii* (= *A. gibbosa*), and *A. stanburyana* (= *A. gibbosa*). Of these, the latter two are now considered synonyms, and I select the remaining one, *A. madagascariensis*, as lectotype of the section.

4. Adansonia gibbosa (A. Cunn.) Guymer ex D. Baum, comb. nov. Basionym: *Capparis gibbosa* A. Cunn. in Heward, J. Bot. British and Foreign 4: 261. 1842. TYPE: Australia. Western Australia: South Grailbourne Island, Careening Cove, *Cunningham 308* (holotype, BM).

Adansonia gregorii Mueller, Hooker's J. Bot. 9: 14. 1857. *Baobabus gregorii* (Mueller) Kuntze, Rev. Gen. Pl. 1: 67. 1891. TYPE: Australia. Northern Territory: Victoria River, *Mueller s.n.* (lectotype, here designated, MEL 229658).

Adansonia rupestris W. Saville-Kent, The Naturalist in Australia, 266–271. 1897. TYPE: Plate V, The Naturalist in Australia (holotype).

Adansonia stanburyana Hochreutiner, Ann. Conserv. Jard. Bot. Genève, 1907/1908: 136. 1908. TYPE: Australia. Western Australia: in the town of Broome outside the police station, *Hochreutiner 2849* (holotype, G).

Small, irregularly shaped, deciduous trees (rarely over 10 m) often with multiple trunks, sometimes having a marked constriction beneath the branches (particularly in the east of its range); crown irregular, primary branches ascending, horizontal or descending. *Bark* smooth, gray. *Leaves* 5–9-foliolate; stipules caducous; leaflets subsessile, elliptic or lanceolate with acute apices and decurrent bases, medial leaflet 8–13(–16) × 2–3(–4) cm, glabrous to pubescent, often pubescent below subglabrous above (especially in east); margins entire. *Flowers* emerging just before or just after leaves; buds erect to horizontal, cylindrical or somewhat wider at the apex than at the base, 10–15 cm long; peduncle 0–2.5 cm; pedicel 2–4(–5.5) cm, longer than the peduncle. *Calyx* lobes (2–)5, 6–9 × 1–1.5 cm, reflexed and twisted at the base of the flower, separating at apex in the morning prior to anthesis; pale green, scabrous to subglabrous outside, cream and villous within; tube 6–10 mm, lacking a well-defined annular swelling. *Petals* white to cream, reflexed for upper 2–4 cm, erect for lower ¾ of length; oblanceolate, approximately 5 times as long as wide (10–15 × 2–3 cm). *Androecium* white to pale yellow, comprising a ± cylindrical staminal tube 3–5 cm × 7–9 mm, surmounted by 170–350 ± erect, free filaments 3–7 cm long. *Ovary* rounded-conical with a thick

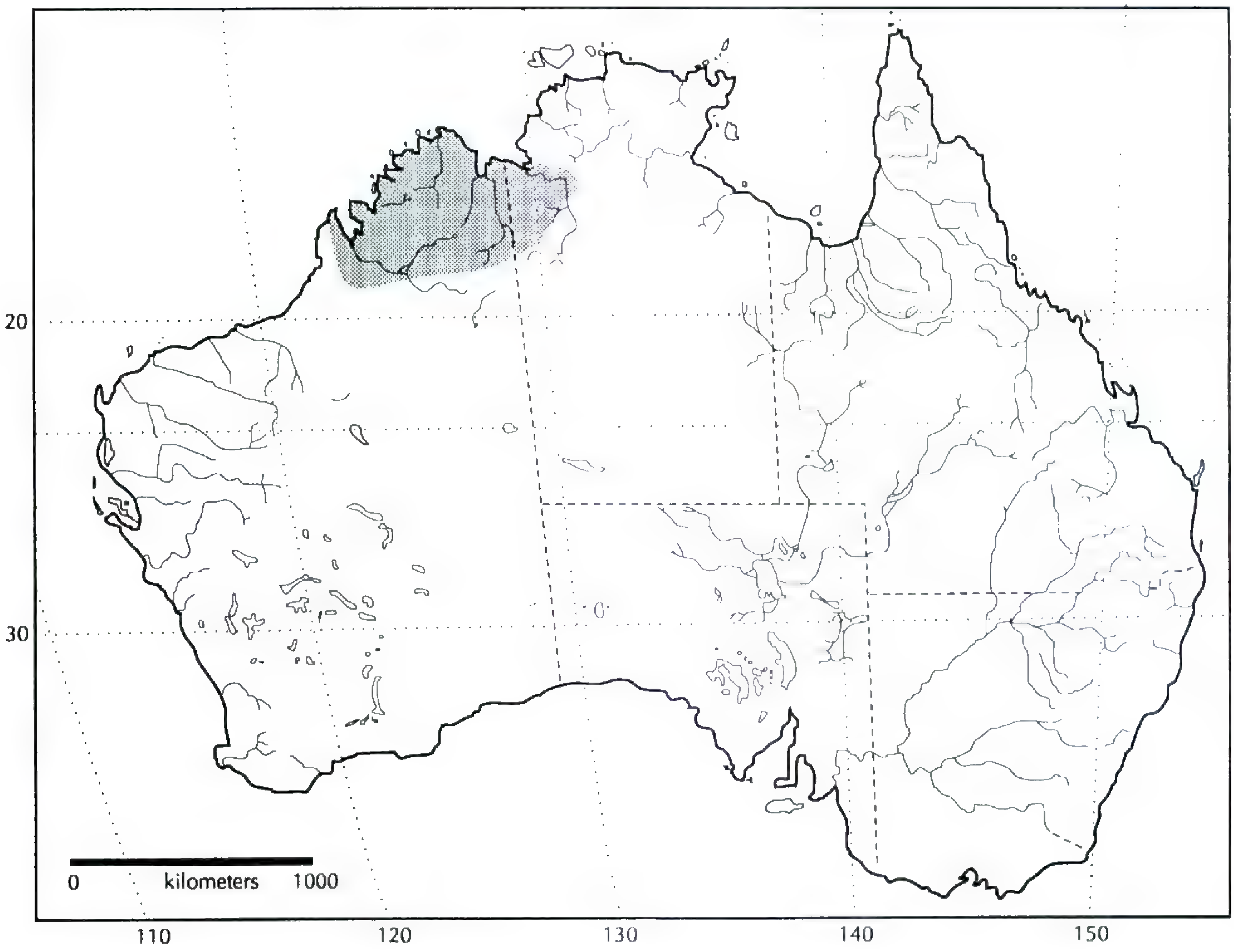

Figure 3. The range of *Adansonia gibbosa* in Australia.

indumentum of brownish upward-pointing hairs. Ovules 300–600. *Style* white, straight, 8–10 cm, not significantly longer than the longest filaments; densely tomentose below, glabrous above, fitting loosely into staminal tube and usually persisting after floral abscission. Stigma lobes spreading, white, browning at tips during the night. *Fruit* globose to ovoid, calyx caducous. Pericarp 3–4 mm thick, brittle, usually cracking while the fruit is still attached to the tree, with few longitudinal fibers and a sparse or dense, brownish or greenish indumentum. Seeds 10–13 × 7–9 × 5–6 mm, reniform, laterally flattened. Germination phanerocotylar.

Representative specimens examined. AUSTRALIA. **Western Australia. West Kimberley:** Oscar Range, close to the Windjana-Tunnel Creek road, 125°17′E, 17°54′S, alt. 200–250 m, 29 Nov. 1989 (l, fl), *Baum 322 & 323* (MO, PERTH); Walcott Inlet, Dec. 1931 (l, fl), *J. R. B. Love s.n.* (PERTH). **North Kimberley:** King Edward River Banks, 24 Nov. 1952 (l, fl), *Broadbent 428* (BM); Port Warrender, Admiralty Gulf, NW Kimberley, 125°50′E, 14°34′S, 22 June 1976 (l), *K. F. Kenneally 5255* (PERTH). **East Kimberley:** alluvial plain between Pentecost and King Rivers, 6.3 km E of Pentecost River on Gibb River road, 125°55′E, 15°51′S, alt. ca. 20 m, 5 Dec. 1989 (l, fl), *Baum 324* (MO, PERTH); 48 mi. NE of Hall's Creek Township, 16 July 1949 (l), *Perry 2501* (CANB, K, NSW, US). **Northern Territory:** banks of Sullivan Creek where it crosses the Victoria highway, 32 km E of Victoria River Crossing, 131°24′E, 15°28′S, alt. ca. 220 m, 8 Dec. 1989 (l, fl), *Baum 325* (MO, PERTH); 2 mi. W of Victoria River, Timber-chalk road, 15 Dec. 1971 (l, fl, fr), *J. Must 904* (BRI, CANB, K, PERTH).

Specimens from naturalized or cultivated plants. AUSTRALIA. **Western Australia:** Broome, in front of police station, 4 Feb. 1905 (l, fl), *Hochreutiner 2849* (G); Broome, outside library, 21 May 1986 (l, fl), *K. F. Kenneally 9733* (PERTH). **Northern Territory:** Darwin (Palmerston), (l, fl), *Mueller s.n.* (G 27).

Distribution (Fig. 3). *Adansonia gibbosa* is restricted to the Kimberley region of north Western Australia and the Victoria River area of northwest Northern Territory. The westernmost limit is roughly at the boundary between the shires of Broome and Derby, 100 km east of Broome (the trees in Broome are all planted). A few individual trees are known from the western shore of King Sound, but only close to the sea (J. Martin, pers.

comm.). These trees may have been dispersed by humans, as they are often located close to aboriginal middens (T. Willing, pers. comm.; Armstrong, 1983). The southernmost individuals are reportedly along Gee Gully (J. Martin, pers. comm.). In the east, the limit appears to coincide with the extent of the Victoria River basin, approximately 32 km east of Victoria River Inn. The northern limit is unclear, but it is likely that throughout most of its range, *A. gibbosa* reaches as far as the coast.

Adansonia gibbosa trees are concentrated around the fringes of rocky ranges, along seasonal creeks, and on the floodplains of the larger rivers. They are absent from the gallery forest along permanent rivers but persist in open areas subject to frequent burning.

Phenology. Leaves November to March. Flowers mid November to mid January, progressing east to west. Fruit ripe May to December.

Cytology. Baker & Baker (1968) reported three separate counts of $2n = 72$, Miège & Burdet (1968) reported $2n = 96$, whereas Baum & Oginuma (1994) found $2n = 88$ from both east and west Kimberley.

Taxonomy and nomenclature. *Adansonia gibbosa* probably arrived in Australia via long-distance dispersal (see above) and, hence, it most likely experienced a severe bottleneck at some point after it separated from the rest of the genus. This suggests that exclusivity has been attained by *A. gibbosa*, as supported by the possession of several diagnostic characters (see key), of which at least one is apomorphic (fruit dehiscence). Furthermore, the current distribution of *A. gibbosa* in northwestern Australia gives no grounds for suspecting long-term genetic isolation of any parts of the species. Thus, I believe that *A. gibbosa* is both exclusive and basal, and hence I recognize it as a species.

The "boab" tree was first discovered by Allan Cunningham, who thought it was a *Capparis.* He left it unnamed in the report of his exploration (Cunningham, 1827), but referred to it as *C. gibbosa* on the specimen and in his diary. Extracts from his diary were published posthumously by Heward (1842), including the description of *C. gibbosa*, constituting a valid publication for that name. The connection between *C. gibbosa* and the Australian baobab (generally refered to as *Adansonia gregorii* Muell.) has been overlooked until recently. However, G. Guymer recognized Cunningham's nomenclatural priority ten years ago and annotated several specimens at Kew and the British Museum "*A. gibbosa* (Cunn.) Guymer." Nonetheless, the new combination had not been published.

The type selected for *Adansonia gregorii* Muell. was annotated with the collection locality "Victoria River," one of the three localities mentioned by Mueller (1857). Other specimens from the same locality are housed at BM and GH; these may or may not constitute isotypes (J. H. Ross, pers. comm.).

Hochreutiner (1908), during a very brief visit to Broome, named a second Australian species, *Adansonia stanburyana*, which was said to differ from *A. gibbosa* in having 5–6 leaflets each about 16 cm long, glabrous below (vs. *A. gibbosa* having 7–9 leaflets of less than 13 cm, tomentose below), a calyx glabrous outside (vs. tomentose in *A. gibbosa*), and a more slender trunk. These traits vary within *A. gibbosa*, but the pattern of variation in no way supports the recognition of more than one species (Ostenfeld, 1918; Wickens, 1983; pers. obs.).

The tree on which Hochreutiner based his description of *Adansonia stanburyana* is located outside the old police station in Broome. Neither its leaves, flowers, nor trunk suggest any deviation from that typical of *A. gibbosa*. The tree bears a plaque indicating that it was planted in 1897, which would have made it about eight years old when Hochreutiner visited Broome in 1905. Thus many of the "distinguishing features" of *A stanburyana* could, in fact, be juvenile traits.

In a travelogue, the naturalist Saville-Kent (1897) referred to the boab tree by the name *Adansonia rupestris*, thereby validly publishing this epithet. He stated that the species is "well exemplified" by plate V; in the absence of any specimens collected by Saville-Kent, or any indications that he consulted any other specimens, this plate is designated holotype.

Ethnobotany. Reported uses of *Adansonia gibbosa* include: fruit eaten as food and anti-scorbutic (e.g., Bennett, 1860; Jackson, 1868; Armstrong, 1983); hollow, water-filled trees and moisture-rich wood used as emergency water supplies (Bennett, 1860; Jackson, 1868; Froggatt, 1934); leaves and the gum exuding from damaged wood eaten (Armstrong, 1983; Boland et al., 1984; Saville-Kent, 1897); bark used to make rope (Armstrong, 1983); and pollen used to make glue (Armstrong, 1983). Furthermore, *A. gibbosa* plays a significant role in aboriginal culture, as illustrated by the large number of trees with carvings and paintings (Crawford, 1968). A bibliography for the Australian baobab (Willis, 1955) gives further references of ethnobotanical interest.

5. Adansonia rubrostipa Jum. & H. Perrier, Mat. Grass. Jan 1909: 8. *Adansonia fony* var. *rubrostipa* (Jum. & H. Perrier) H. Perrier, Notul. Syst., 14: 300. 1952. TYPE: Madagascar. Tsingy de Namoroka (Ambongo), May 1904, *Perrier de la Bâthie 1447* (lectotype, here designated, P).

Adansonia fony Baill. ex H. Perrier, Notul. Syst., 14: 304. 1952 (nom. superfl. based on Baillon's provisional and hence invalid publication, Bull. Mens. Soc. Linn. Paris, 2: 845–846. 1890.). TYPE: Madagascar. Morondava, Apr. 1869, *Grandidier 39* (lectotype, here designated, P).

Small to large deciduous trees (5–20 m) with cylindrical, bottle-shaped, or, rarely, tapering trunks, usually with a distinct constriction beneath the branches. Crown irregular, major branches most often horizontal becoming erect distally, rarely conical spines on upper surfaces of branches. *Bark* usually reddish brown and exfoliating. *Leaves* (3–) 5-foliolate; petioles thin and tapering, 3–7 cm × 0.5–1 mm, glabrous; stipules caducous; leaflets sessile, medial leaflet 4–6(–8) × 1–2 cm, elliptic with acute apices; margin distinctly serrate (teeth ca. 1 mm). *Flowers* emerging when in leaf; buds ± horizontal, elongated-cylindrical, 16–28 cm; flower stalk 1–2.5 cm, green. *Calyx* lobes (3–)5, linear, 15–25 cm × 7–12 mm, reflexed and twisted tightly at the base of the flower, subglabrous, yellowish green with faint, reddish stripes outside, bright red and sparsely villous within. Calyx tube fitting tightly around the petal bases and lacking a distinct annular swelling. *Petals* bright yellow to orange-yellow, spreading, linear with expanded overlapping bases, significantly shorter than the androecium, 12–16 cm × 1.5–2.5 cm. *Androecium* pale yellow, comprising a long, ± cylindrical staminal tube, (3–) 6–10 cm long, 1–1.2 cm diam., surmounted by 100–150 filaments 10–12 cm long, the outer free and spreading, the inner 10–20 erect and united into a central bundle ca. 6 cm beyond the top of the tube. *Ovary* broadly rounded-conical, 7.5 × 9.5 mm, with dense upward-pointing golden hairs. *Style* bright pink, straight, 20–25 cm long, tapering gradually toward stigma; dense upward pointing hairs at base, glabrous above, fitting tightly in staminal tube and falling with it. Stigma red, blackening with age, 5–8 irregular spreading lobes. *Fruit* ± globose, calyx caducous; pericarp 4–5 mm thick with few longitudinal fibers and a dense reddish brown indumentum. Seeds reniform and laterally flattened, size variable, up to 16 × 12 × 8 mm. Germination phanerocotylar.

Representative specimens examined. MADAGASCAR. **Mahajanga Province:** Soalala District: Village of Baly, 4 June 1930 (l, fl), *Decary 7842* (P). Besalampy District: Besalampy, Sep. 1914 (fr), *Perrier de la Bâthie s.n.* (P). Antsalova District: basalt slabs SW of Cap Kimby, Antsalova, 1 Apr. 1966 (l, fl), *SF (Capuron) 24622* (P, TEF). **Toliara Province:** Morondava District: 0.1 km E of Swiss Summer Camp, Kirindy forest, 5 km E of Morondava to Belo-sur-Tsiribihina road, 42 km from Morondava, 44°39′E, 20°02′S, alt. ca. 15 m, 27 Feb. 1989 (l, fl), *Baum 313* (MO, P, TAN). Morombe District: Ambohibitika (Mangoky), Sep. 1911 (l, fl), *Perrier de la Bâthie 8175* (P). Toliara District: 36.6 km N of Toliara, 22 Mar. 1985 (l, fl, imm. fr), *Dorr 4113* (BR, K, MO, P, US); 3 km from La Table towards Toliara, N of road, 21 Mar. 1988 (l, fl), *T. Willing 72* (P). Betioky District: near Lac Tsimanampetsotsa, 14 Feb. 1947 (l, fl), *Humbert 20224* (P); R.N. 10, Soalary, Betioky, 21 Mar. 1953 (fl), *Ravelonanahary 4992* (K, P, TEF). District of Ampanihy: 13 km N of Itampolo on road to Beheloka, 24°34′S, 43°56′E, alt. 100 m, 8 Feb. 1990 (l, fl), *P. B. Philippson 3468* (P).

Distribution (Fig. 4). *Adansonia rubrostipa* extends along the west coast of Madagascar from near Itampolo in the southwest to Soalala in the northwest. It mainly occurs in spiny forest and dry deciduous forest on well-drained calcareous soils and on karstic limestone.

Phenology. Leaves November to April. Flowers February to April (rarely as late as June). Fruit ripe October–November.

Cytology. Miège (1974) reported a chromosome count for *A. rubrostipa* as $2n = 72$, whereas Mangenot & Mangenot (1962) and Baum & Oginuma (1994) found $2n = 88$.

Taxonomy and nomenclature. *Adansonia rubrostipa* is a well-supported exclusive group with a unique combination of characters, including two strong autapomorphies: serrate leaflets and a central bundle of filaments fused beyond the top of the staminal tube. Perrier de la Bâthie (1952a, 1955) recognized two varieties of *A. rubrostipa* (under the name *A. fony*): the northern variety *rubrostipa* and the southern variety *fony*. However, the single feature distinguishing the two varieties, staminal tube length, shows much variation and does not support the recognition of subspecific taxa. In view of the exclusivity of *A. rubrostipa* and the absence of any subdivision, I believe its specific status to be well supported.

The earliest description of *Adansonia rubrostipa* was by Baillon (1890b), who gave it the name *A. fony*: "On le croit bien distinct de l'*A. madagascariensis*, et nous le nommerons, provisoirement, *A. fony*." However, according to the *Code* (Greuter et al., 1988: Art. 34.1b) names suggested provisionally are invalid and, hence, this epithet must be rejected. By the time *A. fony* was validly published (Perrier de la Bâthie, 1952a), Jumelle

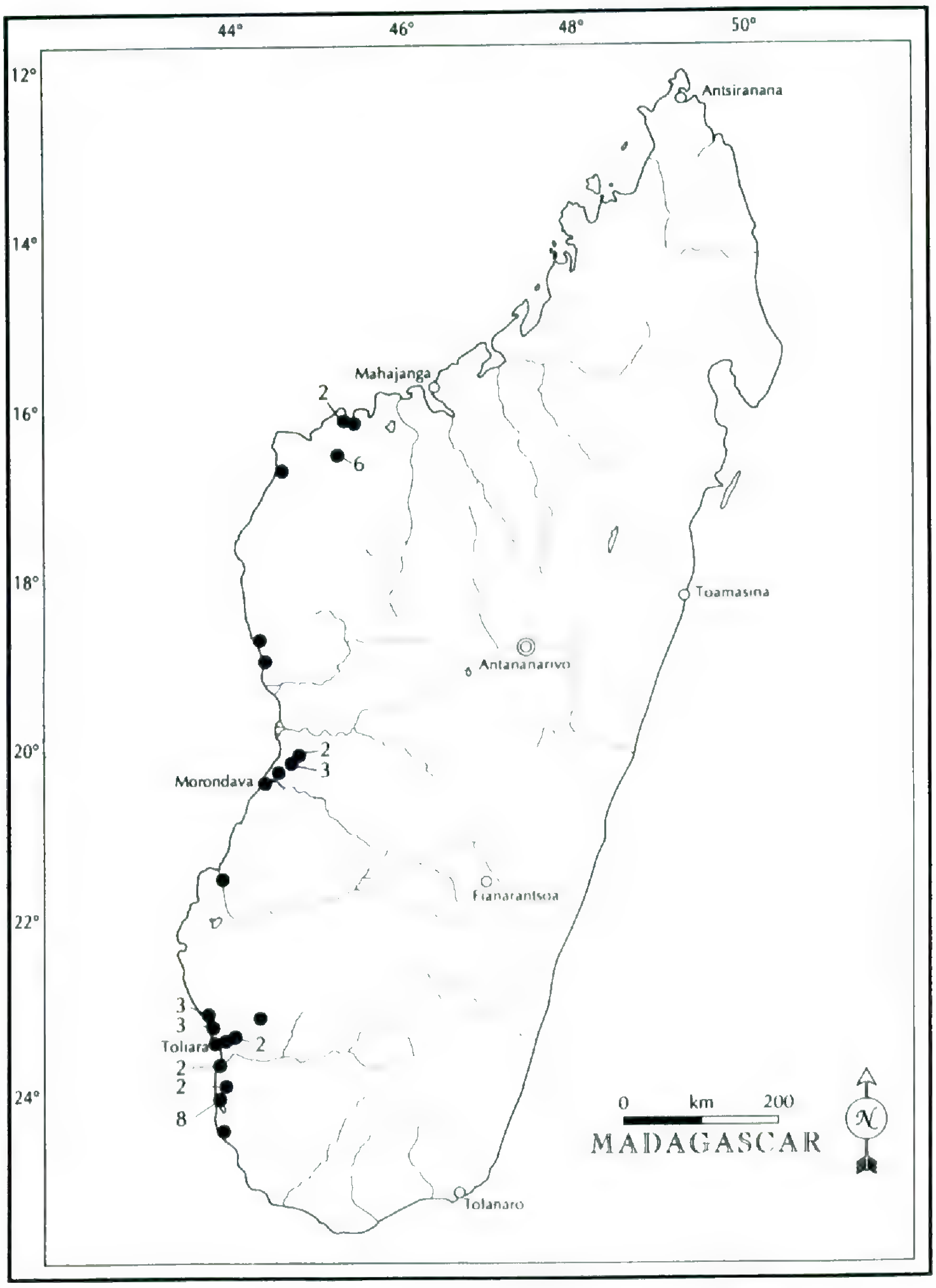

Figure 4. The distribution of *Adansonia rubrostipa* in Madagascar. Collection sites are marked with a circle. When more than one collection is made from the same locality the number of separate collections is indicated.

& Perrier de la Bâthie (1909a) had already published the name *A. rubrostipa*, giving the latter name nomenclatural priority.

Of the two "types" of *Adansonia fony* var. *rubrostipa* mentioned by Perrier de la Bâthie (1955), only *Perrier de la Bâthie 1447* was collected prior to the original 1909 description of *A. rubrostipa*, and this is here designated the lectotype of *A. rubrostipa*. Two other specimens collected by Perrier de la Bâthie prior to 1909 (*1047*, *1447bis*), and almost certainly used for the species description, should be considered syntypes.

Perrier de la Bâthie (1952a) did not indicate types of *Adansonia fony* Baill. ex H. Perrier at the time of publication. Perrier de la Bâthie (1955) designated two "types," *Grevé 38* and *Grandidier 39*. Of these, the latter is the more complete specimen and is here designated lectotype of *A. fony*, with *Grevé 38* being a syntype.

Ethnobotany. *Adansonia rubrostipa* has edible fruits, seeds, and roots (Perrier de la Bâthie, 1952b, 1953), but there is no documentation of them being utilized extensively in Madagascar. In Toliara, *A. rubrostipa* fruits are sometimes sold in the market. They are collected by climbing the small trees, often with the aid of wooden pegs hammered into the trunks. In the vicinity of Morondava, the wood of fire-killed trees is used as thatching (in the same manner as *A. grandidieri*). A species of fungus, with an edible and much sought after fruiting body, is said to grow only on dead *A. rubrostipa* and *A. grandidieri* trunks (Cabanis et al., 1970; pers. obs.). De Bry (in Teil, 1979) mentioned, in the annotation to a drawing that

clearly corresponds to *A. rubrostipa*, that it was used to make canoes. The wood of baobabs is not suited for this use and, hence, this comment is mistaken.

Conservation. In view of its extensive geographical distribution *Adansonia rubrostipa* is conservationally secure, although some populations, such as those 30 km north of Toliara, are threatened by forest destruction for charcoal extraction. In western deciduous forests close to Morondava, *A. rubrostipa* is the dominant tree species and provides an important resource for lemurs (nectar, gum, insects) and insects (sap, nectar, leaves, seeds, and pollen) and probably other animals as well.

6. Adansonia madagascariensis Baillon, Adansonia, 11: 251. 1876. *Baobabus madagascariensis* (Baill.) Kuntze, Rev. Gen. Pl. 1: 67. 1891. *Adansonia bernieri* Baill. ex Poisson, Rech. Fl. Merid. Madag., 20. 1912. TYPE: North Madagascar, *Bernier 2e envoi 364* (lectotype, designated by Perrier de la Bâthie (1955), P; isolectotype, G).

Small to large deciduous trees (5–20 m) with bottle-shaped to cylindrical or tapering trunks and irregular crowns. Primary branches erect, horizontal, or descending. *Bark* smooth, pale gray. *Leaves* 5–7-foliolate; petiole 6–12 cm × 1–2 mm, tapering; stipules caducous; leaflets subsessile with winged petioles, elliptic to oblanceolate with a prominent midrib (markedly raised below when dry), medial leaflet 7–12 × 2–3 cm with 8–16 pairs of irregularly spaced secondary veins, glabrous, apex obtuse to acute; margins entire. *Flowers* produced when in leaf; buds erect to horizontal, elongated-cylindrical, 15–20 cm; flower stalk 2–3 cm, green above, brownish below. *Calyx* lobes linear, up to 18 cm, reflexed and twisted at the base of the flower, green and scabrous outside, dark red and villous within. Calyx tube ca. 2 cm long, fitting tightly around petal bases with a pronounced annular swelling (2–4 mm wide). *Petals* dark red (rarely yellow), linear, approximately 10 times as long as broad, longer than the androecium but usually shorter than the style, ca. 15–20 × 0.8–1.5 cm. *Androecium* pale yellow comprising a cylindrical or tapering tube, 5–6 cm long, 0.6–1 cm diam., surmounted by 90–100 free filaments 7–13 cm long. *Ovary* globose to ovoid, densely covered in brownish upward-pointing hairs. *Style* straight or kinked, dark red, glabrous above with dense upward-pointing hairs below, fitting tightly in the staminal tube and falling with it. Stigma red, lobes irregular, spreading. *Fruit* globose to subglobose, often broader than long (rarely almost ovoid), small (usually less than 10 cm in length), with a sparse indumentum of short brown hairs; pericarp thick (7–9 mm), very tough and woody, with many longitudinal fibers. Seeds distinctly reniform and laterally flattened (10–11 × 7–9 × 4.5–6 mm). Germination phanerocotylar.

Representative specimens examined. MADAGASCAR. **Antsiranana Province:** Antsiranana District: Mahory forest NE of Ankarana, 5 km E of Anivorano to Ambondromifehy road, 49°15′E, 12°52′S, alt. ca. 100 m, 19 Mar. 1989 (l, fl), *Baum 319* (MO, P, TAN); on S side of Antsiranana to Ramena road where it passes the Montagnes des Français, 49°20′E, 12°19′S, alt. 10 m, 23 Mar. 1989 (l, fl), *Baum 320* (MO, P, TAN); Ankarana W of Ambondromifehy, 5 Mar. 1951 (l, fl), *SF (Capuron) 3029* (A, BR, G, K, P, TEF). Vohemar District: Ankarafa (= Ankorefo?), Canton Daraina, 13 May 1955 (l), *SF 14042 (b)* (P, TEF). Ambanja District: Zangoa (= Djangoa) River (l, fl, fr), *Perrier de la Bâthie 5368* (P). **Mahajanga Province:** Maromandia District: Maromandia (Marovato), 23 Mar. 1923 (l, fl, fr), *Decary 1593* (P); near Andranosamonta, May 1909 (l), *Perrier de la Bâthie 8179* (P). Mahajanga District: NE of Mahajanga, 11 June 1940 (fr), *Decary 15343* (P). Ambato-Boeny District: Iabohazo River (of the Bemarivo), Oct. 1907 (fallen fl, fr) *Perrier de la Bâthie 5356* (P).

Cultivated. FRANCE. Paris: seed from Madagascar, 1893 (seedling), *Museum Series F26* (P). MADAGASCAR. **Mahajanga Province:** Marovoay: seeds from Loky, Apr. 1929 (fl, fr), *Perrier 14671* (P).

Material with Uncertain Determination: MADAGASCAR. **Mahajanga Province:** Soalala District: near Soalala, (l), *Decary 7814* (P).

Distribution (Fig. 5). The range of *Adansonia madagascariensis* has been a source of some confusion. Perrier de la Bâthie (1955) suggested that it extends from the very north of Madagascar right down to Ambovombé in the southeast, and his data formed the basis for the maps found in Miège (1974). However, a reassessment of these southern specimens shows that they actually correspond to *A. za*. *Adansonia madagascariensis* thus extends from Antsiranana to the Sambirano region as far as Ankara (Boina region) and perhaps Soalala (Ambongo region) in the northwest.

Adansonia madagascariensis is found in dry or moist deciduous forest on limestone, sandstone, and gneiss. In the vicinity of Antsiranana it often grows within meters of the sea.

Phenology. Leaves present November to April. Flowering February to April. Fruit ripening by November.

Cytology. Miège (1974) reported a chromosome count of $2n = 80–84$; however, this is suspect because the seed came from Morondava, which is

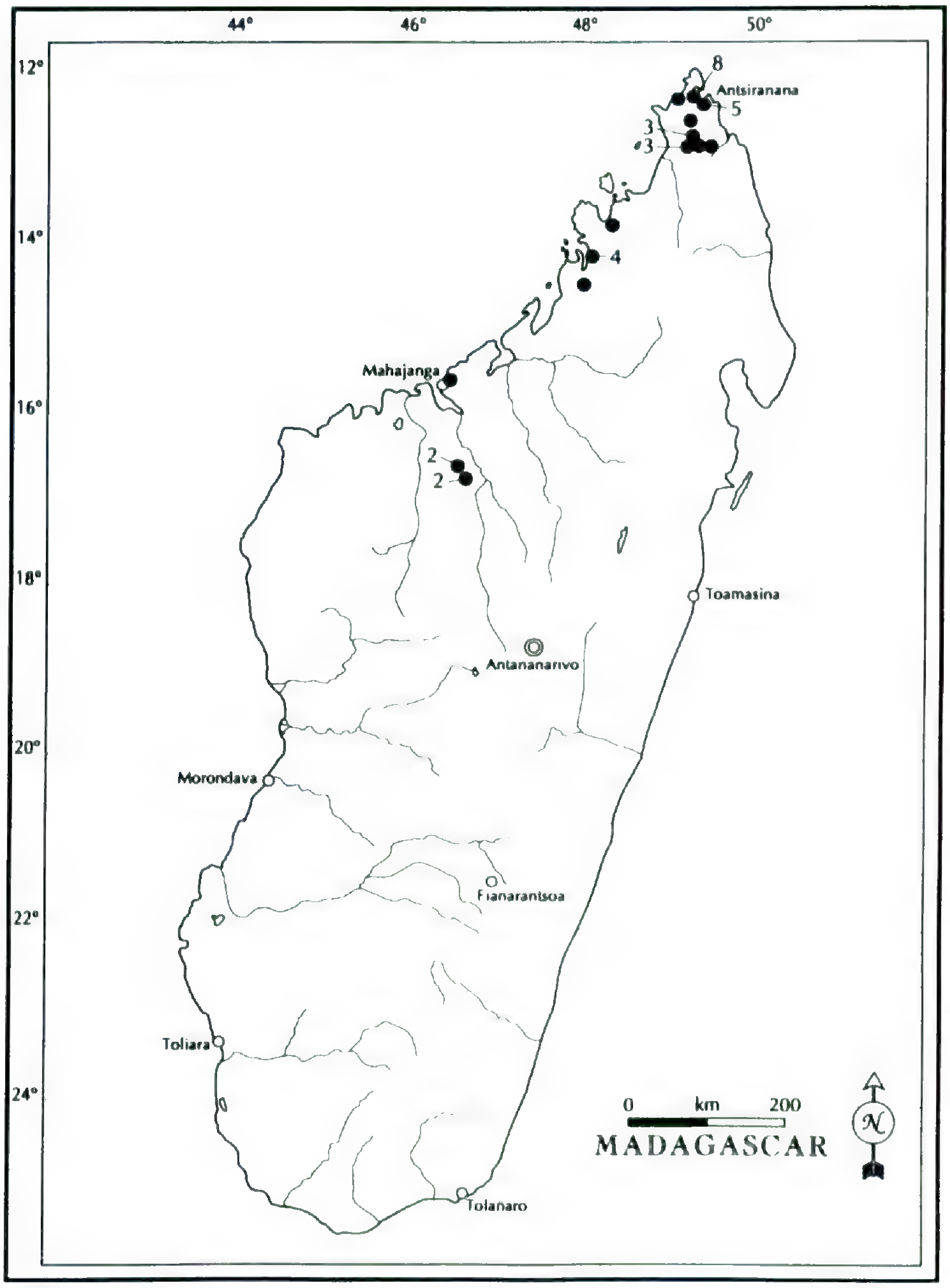

Figure 5. The distribution of *Adansonia madagascariensis* in Madagascar. Collection sites are marked with a circle. When more than one collection is made from the same locality the number of separate collections is indicated.

outside the range of *A. madagascariensis*. Baum & Oginuma (1994) found $2n = 88$.

Taxonomy and nomenclature. *Adansonia madagascariensis* and *A. za* are very similar but are easily distinguished, especially in areas of sympatry (for example, in and around Maromandia), by: petals usually red in *A. madagascariensis* vs. yellow in *A. za*; flowering from February to April vs. November to February; style usually detaching from the ovary during floral abscission vs. usually persistent; fruit globose to subglobose, generally broader than long without a thickened peduncle vs. subglobose to ovoid, usually longer than broad, with a thickened peduncle in the south of its range (see photographs in Jumelle & Perrier de la Bâthie, 1913). Less reliable characters include: ovary globose to ovoid in *A. madagascariensis* vs. conical to ovoid in *A. za*; first 10 or more leaves in seedling simple vs. usually just 1–5 simple leaves; and leaflets with 8–16 secondary veins vs. 10–20.

None of the above characters separating *Adansonia madagascariensis* and *A. za* are diagnostic, i.e., fixed within one taxon and entirely absent from the other (with the possible exception of flowering season). Nonetheless, there are grounds for believing that *A. madagascariensis* is exclusive. The fact that despite the occurrence of sympatry the two species have remained distinct with no evidence of hybridization implies that there is sufficient genetic isolation for barriers to interbreeding ("isolating mechanisms") to have become fixed in the two taxa. Thus, I believe that the best supported hypothesis at this time is that *A. madagascariensis* is exclusive, and in the absence of any indication of genetic subdivision, I treat it as a valid species.

Adansonia madagascariensis was the first Malagasy baobab to be named (Baillon, 1876). The name was misapplied to the fruit of *A. grandidieri* in Baillon (1889), but this error was corrected by Baillon (1893). Poisson (1912) validly published the superfluous name *A. bernieri* based on an annotation by Baillon on the lectotype sheet.

Ethnobotany. The hard dry fruits of *Adansonia madagascariensis* are the least palatable in the genus and, hence, are rarely exploited for food. The swollen roots of young seedlings are edible and reportedly make an excellent vegetable (Perrier de la Bâthie, 1952b). No other uses are reported, although Perrier de la Bâthie (1953) suggested that, in view of its prodigious growth rate (12–15 m and 40–60 cm diam. after 12 years), it could be exploited for cellulose. However, his claim that at 24 years the trees had attained 40 m is probably a typographical error.

Conservation. The conservational status of *Adansonia madagascariensis* is unknown, although it is clearly less threatened than the other northern species, *A. perrieri* and *A. suarezensis.*

7. Adansonia za Baill., Bull. Mens. Soc. Linn. Paris, 2: 844. 1890. TYPE: Madagascar. Mahafaly plateau, *Grevé 37* (lectotype, designated by Perrier de la Bâthie (1952a), P).

Adansonia za var. *boinensis* H. Perrier, Notul. Syst., 14: 304. 1952. TYPE: Madagascar. Ankara, Kamakama, Oct. 1902, *Perrier de la Bâthie 960A* (lectotype, here designated, P).

Adansonia bozy Jum. & H. Perrier, Ann. Mus. Colon. Marseille, 18: 447–451. 1910. *Adansonia za* Baill. var. *bozy* (Jum. & H. Perrier) H. Perrier, Notul. Syst., 14: 304. 1952 (= *Adansonia za* var. *bozo*). TYPE: Madagascar. Andranomandevo, Sambirano valley, Jan. 1909, *Perrier de la Bâthie 8159* (holotype, P).

Adansonia alba Jum. & H. Perrier, Matières Grasses, 1909: 1511. 1909. TYPE: Madagascar. Upper basin of the Andranomalaza to the N of Bezofo (Bejofo), in dense forest by a waterfall close to 500 m, on rocks (syenite), Oct. 1908, *Perrier de la Bâthie 8166* (holotype, P).

Usually tall (up to 30 m), rarely stunted (less than 5 m when mature), deciduous trees with usually single, cylindrical or slightly tapering trunks, often with irregular swellings; crowns rounded; primary branches usually ascending and tapering. *Bark* gray and ± smooth. *Leaves* 5–8-foliolate; petiole 5–15 cm × 1–4 mm; stipules caducous; leaflets sessile to long-petiolulate (up to 3 cm), broadly elliptic to lanceolate, apex usually apiculate, rarely mucronate; medial leaflets up to 20 × 8 cm in the north, usually less than 10 × 4 cm in the south, with 10–20 pairs of regularly spaced secondary veins (more in north), usually alternating with prominent intersecondaries, glabrous or somewhat scabrous; margins entire. *Flowers* emerging simultaneously or soon after the leaves; buds erect to horizontal, elongated-cylindrical, 15–24 cm × 1.5–2.5 cm; flower stalk 2–3 cm, green. *Calyx* lobes (3–)5, reflexed and twisted at the base of the flower, 15–22 cm × 10–12 mm, green and scabrous outside, dark red villous within. Calyx tube fitting tightly around the petal bases, with a marked annular swelling (ca. 2 mm wide). *Petals* yellow, sometimes with a diffuse reddish medial streak on the adaxial surface, linear, at least 10 times as long as wide, 14–24 cm × 10–16 mm, exceeding the style and androecium. *Androecium* pale yellow, comprising a long cylindrical or tapering staminal tube, 4–6.5 cm, surmounted by 100–120 free filaments, up to 12 cm long. *Ovary* conical to ovoid, covered in dense, upward-pointed hairs. *Style* dark red, straight, 16–22 cm glabrous above, dense, upward-pointing hairs below, usually fitting loosely in the staminal tube and persisting after floral abscission (rarely to fruit). Stigma red, 3–5 mm diam. with irregular lobes. *Fruit* subglobose, oblong or ovoid, 10–30 cm long, 6–15 cm wide, rarely curved; usually with longitudinal ridges and, except in the north, a distinctly swollen peduncle. Pericarp thick and tough with many longitudinal fibers, usually blackish with a sparse indumentum. Seeds reniform, laterally flattened, variable in size, up to 12 × 11 × 8 mm. Germination phanerocotylar.

Representative specimens examined. MADAGASCAR. **Antsiranana Province:** Ambanja District: Ambodibozo near Ambanja, alt. ca. 5 m, Mar. 1951 (l, fr), *Humbert & Capuron 25500* (K, P). **Mahajanga Province:** Maromandia District: Antanandava I village, ca. 11 km E of Maromandia, isolated tree ca. 1.5 km W of village, 14°16′S, 48°08′E, 1 Nov. 1991 (l, fl), *Baum 332* (MO, P, TAN); Ambondrona village (ca. 2 km E of Ambaibo), 500 m E of village near the Andranomaiaza river, 14°13′30″S, 48°22′30″E, alt. ca. 100 m, 4 Nov. 1991 (l, fl), *Baum 336* (MO, P, TAN). Marovoay District: Ankaboka near Marovoay, Dec. 1909 (l, fl), *Perrier de la Bâthie 8189* (BR, G, P). Ambato-Boeny District: Ankara, Kamakama, Oct. 1902 (l, fl, fr), *Perrier de la Bâthie 960A & 960C* (P). Kandreho District: Kariza forest on Kelifely tablelands, Nov. 1904 (fl), *Perrier de la Bâthie 960E* (P). Maevatanana District: Andriba, isolated trees at foot of Mount Andriba on Gneiss, 17 Jan. 1942 (l, fl), *Decary 17088* (P). **Toliara Province:** Morondava District: Kirindy forest, 4.5 km E of Morondava to Belo-sur-Tsiribihina road 42 km from Morondava, 50 m from the Kirindy river bed, 20°02′S, 44°39′E, alt. 25 m, 24 Dec. 1991 (l, fl), *Baum 344* (MO, P, TAN); near Analaiva, E of Morondava, 28 Dec. 1962 (l, fl), *SF (Capuron) 22141* (A, BR, G, K, MO, P, TEF). Mahabo District: 21 km E of Mahabo, 4 Dec. 1920 (fl), *Keraudren-Aymonin & Aymonin 25917* (P). Miandrivazo Dis-

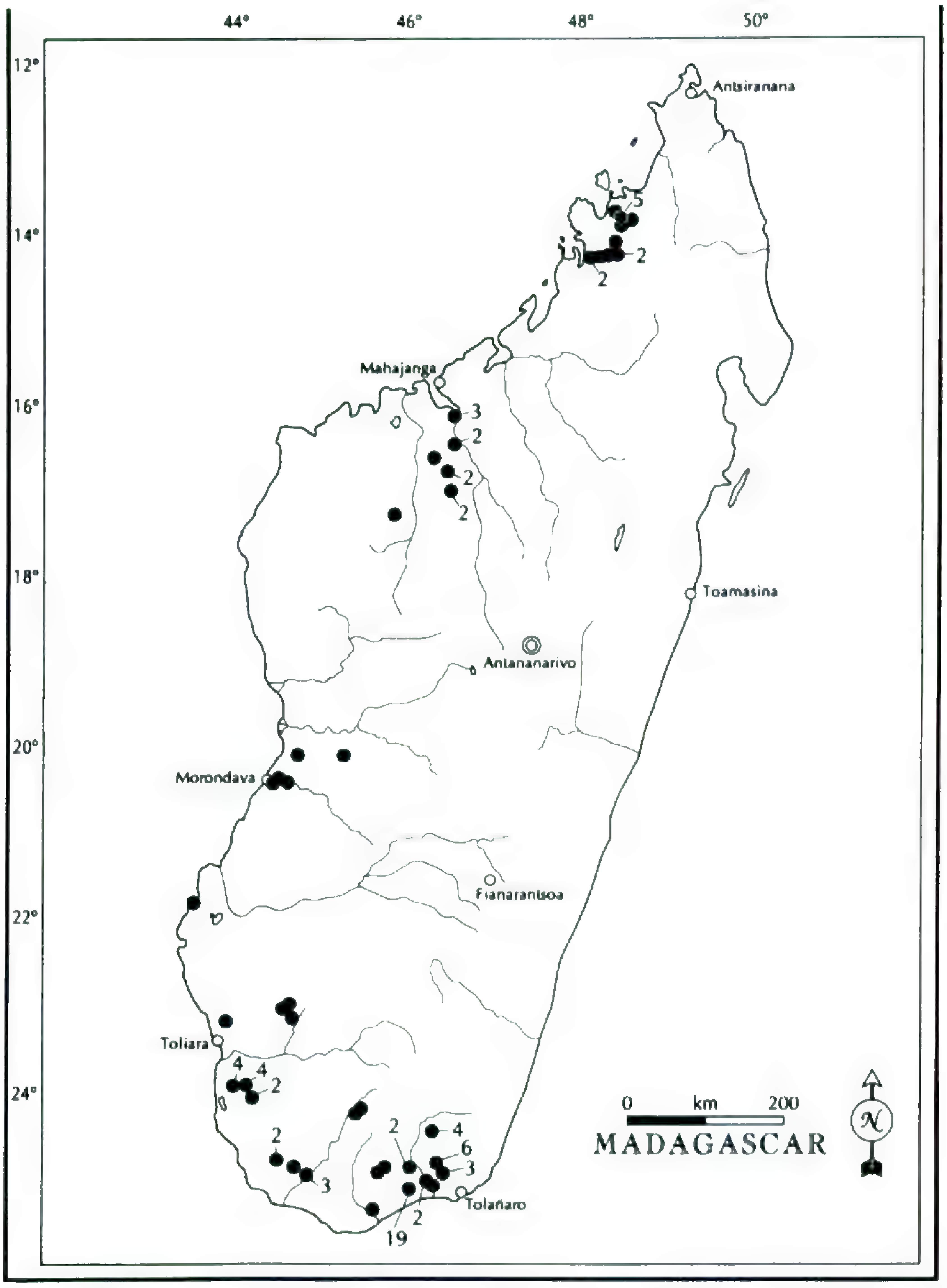

Figure 6. The distribution of *Adansonia za* in Madagascar. Collection sites are marked with a circle. When more than one collection is made from the same locality the number of separate collections is indicated.

trict: Sakeny River, Aug. 1910 (l, fl), *Perrier de la Bâthie 8180* (P). Morombe District: Morombe, Oct. 1940 (fr), *Decary 16426* (P). Sakaraha District: Analamarina, SE of Sakaraha, alt. 500 m, 28 Dec. 1961 (l, fl), *SF (Capuron) 20595* (BR, G, MO, P, TEF). Toliara District: limestone plane of the basin of the Fiherana, Jan. 1910 (l), *Perrier de la Bâthie 8182* (P). Betioky District: near Beza Mahafaly reserve, near Betioky, valley of the Ehazoara river, E of Sakamena, 23°40'S, 44°39'E, alt. 170 m, 29 Nov. 1987 (l, fl), *P. B. Philippson 2638* (MO, P). Ampanihy District: between Beloha and Ampanihy, 19 Feb. 1949 (l, fl), *SF 455* (P, TEF). Bekily District: Ambatofotsy, 15 Jan. 1957 (l, fl), *SF 3376* (P, TEF). Ambovombe District: Ambovombe, 24 Jan 1925 (fl, l), *Decary 3554* (BR, G, P, TAN). Amboasary District: Fangidraty NE Androy, 21 Nov. 1931 (l, fl), *Decary 9319* (K, P); Andohahela parcelle 2, NE of Amboasary near Hazafotsy, 24°50'S, 46°32'E, 100 m, 12 Dec. 1991 (l, fl), *S. T. Malcomber 1141* (MO, P).

Cultivated. FRANCE. Paris, 1893 (seedling) *Baillon s.n.* (P). MADAGASCAR. Jardin Tsimbazaza, 7 May 1941 (l), *Jardin Tsimbazaza 4896* (TAN).

Immature or incomplete material with uncertain determinations. Near Radama [= Sahamalaza], Maromandia, 11 Oct. 1922 (imm. l), *Decary 1146* (P); N of Fiherana, dry forest of Fandrare, 40 km N of Tulear, Mar. 1934 (juv. shoot), *Humbert 14348bis* (P); Besevo, Oct. 1899 (l), *Perrier de la Bâthie 960* (P); Ambolibozo, Sambirano May 1924 (seedlings), *Perrier de la Bâthie 16340a* (P).

Distribution (Fig. 6). *Adansonia za* occurs in dry deciduous forest, spiny forest, savannas, and scrubland from Andohahela and the Mandrare River in the southwest through southern and western Madagascar to the Boina region and the Sambirano River basin. In some deciduous forests in the south it constitutes the dominant tree species (e.g., Analava west of Betioky and to a lesser extent Sakaraha and the forests east of Ankilizato). In the

northwest it is less abundant, concentrated near rivers.

Phenology. In leaf throughout the wet season. Flowering November to February, earlier in the north, later in the south. Ripe fruit from end of dry season.

Cytology. Miège (1974) reported $2n = 48$ for specimens collected in the southwest of Madagascar. Baum & Oginuma (1994) found $2n = 88$ from both south and north Madagascar.

Taxonomy and nomenclature. As discussed above, there are few fixed differences between *Adansonia za* and *A. madagascariensis.* The case for exclusivity is weaker in *A. za* than it was in *A. madagascariensis* in view of its wide geographical range and the fact that some individuals in the north share features with *A. madagascariensis* that are absent from southern individuals assigned to *A. za.* One possibility is that *A. za* is a "metaspecies" (Donoghue, 1985), i.e., that the organisms constituting *A. za* are not members of any exclusive species. This situation could have arisen, for example, under the following scenario, which assumes for the sake of illustration that *A. madagascariensis* and *A. za* are sister groups. (1) A small subset of the common ancestral taxon became genetically isolated. (2) This subset continued to have a small population size and/or went through a severe bottleneck, becoming the exclusive species *A. madagascariensis.* (3) The remainder of the common ancestor, constituting the putative taxon "*A. za,*" retained a large population size and did not become exclusive. If this scenario were correct, the organisms in "*A. za*" would not be in any basal, exclusive group, because the smallest exclusive group that they are members of is *A. madagascariensis* + *A. za,* which contains the exclusive group *A. madagascariensis.* We do not know if such a series of events occurred, and thus we cannot determine if *A. za* is a species or a metaspecies. Given this uncertainty, what is the prudent course? In the absence of guidelines for dealing with metaspecies in a taxonomy, and in order to avoid unecessarily destabilizing the taxonomy of *Adansonia,* I here advocate recognition of *A. za* as a species, while flagging it as a taxon worthy of more detailed analysis.

Perrier de la Bâthie (1955) proposed an infraspecific classification of *Adansonia za,* recognizing three varieties. Although there is significant variation from the north to the south of the range of *A. za,* this variation is gradual and I see no value in recognizing subspecific taxa. The southern populations of *A. za* are the most distinctive because of their long-petiolulate leaflets and ovoid-cylindrical fruit with swollen peduncles. These characters are only gradually replaced by sessile leaflets and normal peduncles as one goes north. Some other characters also vary clinally, such as leaflet size (larger in north) and flowering season (earlier in north), but again I see no use in forcing a continuum of variation into discrete taxa.

There is some nomenclatural confusion as to the taxon to which the name *Adansonia za* should apply. Hochreutiner (1908) pointed out that the description given by Baillon (1890b) contradicts the illustration labeled "*A. za*" (79C) in *Histoire Naturelle des Plantes* (Baillon, 1893) and the description given by Drake del Castillo (1902). Hochreutiner concluded that the plate predated Baillon's description and hence had priority. However, Jumelle & Perrier de la Bâthie (1910) pointed out that although plates 79A and 79B predate Baillon (1890b), the others, 79C to 79I, were not issued until 1893, and hence the written description has priority. Of the three plates (79C, D, and I) labeled *A. za,* two (79C and I) appear to be *A. suarezensis,* whereas plate 79D is an accurate representation of the fruit of *A. za* Baill. Thus, the original description of *A. za* is Baillon (1890b). The lectotype (*Grevé 37*) was selected by Perrier de la Bâthie (1952a), but he incorrectly gave the collection locality as Morondava.

The first mention of *Adansonia bozy* is in Jumelle & Perrier de la Bâthie (1909b), but the taxon was only recognized provisionally and hence this name was not validly published until 1910 (Jumelle & Perrier de la Bâthie, 1910). Perrier de la Bâthie (1952a) subsumed this taxon as a variety of *A. za.* However, in this publication Perrier de la Bâthie used the spelling *bozo,* an error which was corrected by Perrier de la Bâthie (1955). Only one specimen of *A. za* var. *bozy* was collected early enough to have been consulted by Jumelle & Perrier de la Bâthie (1910), and this is, therefore, assumed to be the holotype.

In selecting a lectotype for *Adansonia za* var. *boinensis* I chose *Perrier de la Bâthie 960A,* the most complete specimen from one of the localities mentioned by Jumelle & Perrier de la Bâthie (1909a, 1910) that predates these articles.

The putative species *Adansonia alba* was named by Jumelle & Perrier de la Bâthie (1909a) to accommodate a specimen Perrier de la Bâthie collected along the Andranomalaza River. This species was said to be diagnosed by its white flowers and short staminal tube. In the course of my observations and collections along the Andranomalaza

River, all the baobabs I encountered could be assigned to *A. za*. Furthermore, the type specimen, though of poor quality, shows no features that distinguish it from *A. za*. Thus, I believe that *A. alba* is synonymous with *A. za*. The characters that supposedly separate the species, white petals and short staminal tube, were probably incorrectly scored by Perrier de la Bâthie, being based upon fallen flowers.

Ethnobotany. *Adansonia za* has several common names, for example, "za" or "zabe" in the south and "bozy" or "bozybe" in the north, and "ringy" or "boringy" in the Ambongo region (Perrier de la Bâthie, 1955).

Little is known about the human exploitation of *Adansonia za*, but Jumelle & Perrier de la Bâthie (1912) reported that the seeds are eaten and the trunk is sometimes hollowed out as a cistern for storing water. Perrier de la Bâthie (1952b) mentioned that the seedling roots are an edible vegetable, and Miège (1974) reported that *A. za* is destroyed by ranchers so that their cattle can feed on the moist wood.

Conservation. In view of the extensive geographical range, *Adansonia za* is conservationally secure, despite the fact that several local populations are endangered by forest clearance. *Adansonia za* has diverse interactions with animals: it provides nesting sites for birds, holes for carnivores and lemurs (M. Nicoll, pers. comm.); perches for territorial surveillance and display by birds and the sportive lemur (*Lepilemur* sp.); many insects feed on the leaves, sap, nectar, and pollen; sunbirds (*Nectarinia souimanga*) feed on nectar; sifaka (*Propithecus verrauxi verrauxi*) feed on flower buds; and fork-marked lemurs (*Phaner furcifer*) feed on exuded gum (Petter et al., 1975).

8. Adansonia perrieri Capuron, Notul. Syst. (Paris) 16: 66. 1960. TYPE: North Madagascar. Ankarana Plateau E of Ambondromifehy, valley on clay and limestone, 13 Nov. 1958, *SF (Capuron) 19000* (holotype, P; isotype, TEF).

Medium to tall deciduous trees (up to 30 m) with ± cylindrical trunks and irregular crowns. Major branches horizontal or ascending at about 45°. *Bark* smooth, pale gray. *Leaves* 5–11-foliolate (usually over 9 on reproductive shoots); petioles sturdy, usually pubescent; stipules triangular or linear, up to 15 mm, persistent; leaflets sessile or subsessile (petiolule up to 5 mm long on vegetative shoots), medial leaflet 8–12 × 3–4.5 cm, elliptic-obovate, with 15–24 pairs of secondary veins on reproductive shoots; apex acute to apiculate, pubescent (subglabrous on vegetative shoots). *Flowers* emerging at or just before leaf emergence; buds erect to horizontal, elongated-cylindrical; flower stalk ca. 2 cm long, yellowish green, pubescent. *Calyx* lobes (3–)5, 13–18 cm × 8–11 mm, reflexed and twisted at the base of the flower; green scabrous outside, cream or pinkish and villous within. Calyx tube fitting tightly around the petal bases and lacking a marked annular swelling. *Petals* pale yellow, becoming darker with age, oblong, 15–23 cm × 35–45 mm, approximately 5 times as long as broad. *Androecium* pale yellow, comprising a very long and narrow tube tapering from bottom to top, 13–20 cm long, 3–13 mm diam., surmounted by 180–220 spreading, free filaments, 1.0–2.2 cm. *Ovary* conical to ovoid, with dense upward-pointing hairs. *Style* red, at least distally, 16–22 cm, exceeding staminal tube by 2–4 cm, glabrous, fitting tightly into the staminal tube and falling with it at anthesis. Stigma red or pink, 4–8 mm diam., irregularly lobed. *Fruit* broadly ovoid to oblong, up to 25 cm long, 1.5–2.5 times as long as broad. Pericarp 8–9 mm thick and tough with many longitudinal fibers, indumentum of short, brown, clumped hairs. Seeds reniform, laterally flattened, 9–11 × 8–9 × 5–6 mm. Germination phanerocotylar.

Specimens examined. NORTH MADAGASCAR. **Antsiranana Province:** Antsiranana District: 7 km N of Station Roussette where the trail to Andranonaomby crosses the Makis River, Parc National de Montagne d'Ambre, 49°10'E, 12°29'S, alt. 650 m, 18 Nov. 1988 (fl), *Baum 308* (MO); 1 km W of Beanamalao on the banks of the Andranofanjava River where it is crossed by the trail from Station Rousette to Andranofanjava village, 49°05'E, 12°31.5'S, alt. 475 m, 11 Mar. 1989 (l), *Baum 314* (MO); 7 km N of Station Roussette where the trail to Ankorefo crosses the Makis River, Parc National de Montagne d'Ambre, 49°10'30"E, 12°29'S, alt. 650 m, 4 Dec. 1991 (l, fl), *Baum 340 & 341* (MO, P, TAN); Montagne d'Ambre, left bank of Makis River by the path from Joffreville to Andranofanjava, 21 Nov. 1958 (l, fl), *SF (Capuron) 20076* (G, P, TEF); Camp d'Ambre, Jan. 1926 (fr), *Humbert 17560* (P); S of Antsiranana on main highway, streamside, 12°40'S, 49°15'E, 400 m, 24 Nov. 1989 (fl, l), *McPherson 14538* (MO, P); near Montagne d'Ambre, *Perrier de la Bâthie 17560* (P). Ambilobe District: limestone plateau of Ankarana to the NE of Ambondromifehy, 13 Nov. 1958 (l, fl), *SF (Capuron) 19000* (K, MO, P, TEF); Ankarana, 200–350 m, 4–9 Mar. 1951 (l, fr), *Humbert & Capuron 25680* (P); Ankarana Plateau, dry valley E of Ambondromifehy on limestone and basalt outcrops, 6 Mar. 1951 (l, fr), *SF 3038* (P, TEF). **Toamasina Province:** Ambatondrazaka District: Lac Alaotra (cultivated?), 1000 m, 1945 (l), *Perrier de la Bâthie 17560bis* (P).

Figure 7. The distribution of *Adansonia perrieri* in northern Madagascar. Collection sites are marked with a circle. When more than one collection is made from the same locality the number of separate collections is indicated. Some additional localities known by the author are marked with a triangle.

Distribution (Fig. 7). *Adansonia perrieri* is known from only five sites. The population at Montagne d'Ambre is at about 650 m in evergreen rainforest on the banks of the Rivière des Makis and comprises about 12 individuals. The Ambondromifehy population is made up of many individuals (even hundreds; Capuron, 1960) scattered in dry deciduous forest. The remaining three localities comprise three or fewer individuals (one of them, Mahory forest, eastern Ankarana, is not represented by a collection). Other populations certainly remain to be discovered in the region of Ankarana and Montagne d'Ambre.

Phenology. Leaves throughout wet season (November to April). Flowering November to December. Fruit ripe in October and November.

Cytology. Miège (1974) reported $2n = 96$. Baum & Oginuma (1994) found $2n = 88$.

Taxonomy and nomenclature. In view of its limited geographic range, the possession of several unique characters, some of which are apomorphic (e.g., persistent stipules, extremely long, narrow staminal tube), and in the absence of intraspecific variation, *Adansonia perrieri* is a well-supported species.

Ethnobotany. *Adansonia perrieri* shares its common name, "bozy," with other species of *Adansonia* occurring in northern Madagascar. Fruit is collected for the edible pulp, but no other uses are reported.

Conservation. Although it is quite likely that *Adansonia perrieri* is more widespread than now thought, only one of four sites is populated by more than a handful of individuals. All the populations except at Montagne d'Ambre are significantly disturbed by humans, especially through fire and the extraction of wood for charcoal and timber. Even in Montagne d'Ambre the long-term survival of *A. perrieri* is threatened due to seed predation by introduced rats. Despite the extensive production of fruit, very few remain intact, and only those that fall directly into the river have a good chance of escaping predation. In view of its rarity and the various threats it faces, *A. perrieri* is perhaps the most endangered Malagasy baobab.

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List of Species

1. *Adansonia grandidieri* Baill.
2. *Adansonia suarezensis* H. Perrier
3. *Adansonia digitata* L.
4. *Adansonia gibbosa* (A. Cunn.) Guymer ex D. Baum
5. *Adansonia rubrostipa* Jum. & H. Perrier
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STATISTICAL SUMMARY OF SOME OF THE ACTIVITIES IN THE MISSOURI BOTANICAL GARDEN HERBARIUM, 1994

	Vascular	Bryophyte	Total
Acquisition of Specimens			
Staff Collections (includes bryophytes)	27,518	—	27,518
Purchase	211	10,000	10,211
Exchange	42,688	2,150	44,838
Gifts	8,103	55	8,158
Total acquisitions	78,520	12,205	90,725
Mountings			
Newly mounted	79,736	21,304	101,040
Mounted when received	84,000*	0	84,000
Total specimens filed	163,736	21,304	185,040
Repairs			
Specimens repaired	30,556	n/a	30,556
Specimens stamped	5,784	n/a	5,784
Total repairs	36,340	0	36,340
Specimens sent			
On exchange	29,828	4,824	34,652
As gifts	28,892	1,539	30,431
Total	58,720	6,363	65,083
Loans sent			
Total transactions	680	33	713
Total specimens	29,307	2,551	31,858
To U.S. institutions			
Transactions	407	27	434
Specimens	16,084	2,368	18,452
To foreign institutions			
Transactions	273	6	279
Specimens	13,223	183	13,406
To student investigators			
Transactions	66	7	73
Specimens	6,633	822	7,455
To professional investigators			
Transactions	614	26	640
Specimens	22,674	1,729	24,403
Loans received			
Transactions	321	22	343
Specimens	16,697	1,818	18,515

* The 84,000 vascular plants "mounted when received" are all specimens of Chinese plants purchased directly from China.

	From U.S.	From abroad	Total
Visitors	427	123	550

On 31 December 1994 the total number of mounted, accessioned specimens in the herbarium was 4,488,028 (4,225,255 vascular plants and 262,773 bryophytes). Corrected total figures for 1993 are: 4,297,204 total mounted, accessioned specimens in the herbarium (4,055,735 vascular plants and 241,469 bryophytes).—David Brunner, *Curator of the Herbarium.*

NOTICE

THE RUPERT BARNEBY AWARD

The New York Botanical Garden is pleased to announce that Ing. Nelson Zamora, of the Instituto Nacional de Biodiversidad (INBio), Costa Rica, is the recipient of the 1994 Rupert Barneby Award. Ing. Zamora will be working on several groups of mimosoid and caesalpinioid legumes for Costa Rica.

The New York Botanical Garden also invites applications for the 1995 Rupert Barneby Award. The award of $1,000.00 is to assist researchers planning to come to The New York Botanical Garden to study the rich collection of Leguminosae. Anyone interested in applying for the award should submit their curriculum vitae, a letter describing the project for which the award is sought, and how the collection at NYBG will benefit their research. Travel to NYBG should be planned between January 1, 1996 and January 31, 1997. The letter should be addressed to Dr. Enrique Forero, Director, Institute of Systematic Botany, The New York Botanical Garden, Bronx, NY 10458-5126 USA, and received no later than December 1, 1995. Announcement of the recipient will be made by December 15th. Anyone interested in making a contribution to THE RUPERT BARNEBY FUND IN LEGUME SYSTEMATICS, which supports this award, may send their check, payable to The New York Botanical Garden, to Dr. Forero.

Volume 82, Number 3, pp. 355 472 of the ANNALS OF THE MISSOURI BOTANICAL GARDEN
was published on July 31, 1995.

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Annals of the Missouri Botanical Garden, Volume 82, Number 2: Alternative Genes for Phylogenetic Reconstruction in Plants

A symposium cosponsored by the American Society of Plant Taxonomists and the Botanical Society of America, organized by Pamela S. Soltis and Douglas E. Soltis, and presented at the 1993 AIBS meetings.

Although the chloroplast gene *rbc*L has been successfully used to reconstruct plant phylogeny, many important questions of plant phylogeny and evolution cannot be addressed using it. The contributors to this issue of the *Annals* explore the potential of eight alternative genes or DNA regions for phylogenetic reconstruction at a variety of hierarchical levels. Both nuclear and chloroplast genes are evaluated. Three regions of the nuclear ribosomal RNA cistron are explored: the 18S gene, the internal transcribed spacers (ITS), and the 26S gene. Small multigene families from the nuclear genome may also carry phylogenetic signal: the phytochrome gene family and the small heat shock gene family. Three genes from the chloroplast genome are also considered: *atp*B, *ndh*F, and *mat*K. Each paper describes the location, size, structure, and rate of evolution of the chosen gene and discusses its potential for phylogenetic study. This issue also contains: "The Comparative Pollination and Floral Biology of Baobabs (*Adansonia*–Bombacaceae)" by David A. Baum and "In Memoriam: *Peter G. Martin*." Annals 82(2) 1995. 174 pages. $27.50 U.S. $28.00 Non-U.S.

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Missouri Botanical Garden

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This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

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Annals of the Missouri Botanical Garden

THE BIOLOGY AND SYSTEMATICS OF *FUCHSIA* IN THE SOUTH PACIFIC[1]

Eric J. Godley[2] *and Paul E. Berry*[3]

ABSTRACT

There are only four species of *Fuchsia* that are not native to the New World, three occurring on New Zealand and the fourth on Tahiti. The biology and systematics of these taxa are examined here in detail, including known and new information on phenology, habitats, pollination, dispersal, cytology, hybridization, and evolutionary relationships. Because of the basal position of *F. procumbens* in the clade of South Pacific fuchsias and its large suite of unique characters, it is recognized as a new section, *Procumbentes*, within the genus. This leaves *F. cyrtandroides*, *F. excorticata*, and *F. perscandens* in section *Skinnera*. *Fuchsia* ×*colensoi* is treated here as a widespread and variable hybrid between *F. excorticata* and *F. perscandens*. Keys and descriptions of the taxa are provided.

The genus *Fuchsia* of the family Onagraceae is almost entirely confined to South and Central America. Ranging from Tierra del Fuego north to Mexico, there are nearly 100 species currently recognized in 9 sections (Berry et al., 1988). The occurrence therefore of a group of four species on a few islands of the Southwest Pacific is of considerable phytogeographical interest. Although these species have been treated as comprising the single South Pacific section *Skinnera*, they present a greater diversity of habit than any other section in the genus. The large tree habit in the widespread

[1] We thank Diane H. Smith for her technical assistance to E. Godley, particularly with the artificial crosses. We also thank Peter H. Raven for travel support provided to P. Berry through National Science Foundation grant BSR-890648 and for making available personal notes on fieldwork he conducted on the New Zealand fuchsias in 1969 and 1970. The D.S.I.R. of New Zealand provided an overseas travel grant for E. Godley to visit Tahiti, where Maurice Jay provided him valuable logistic support. Plant material from the island was kindly sent to E. Godley by R. Millaud and J. N. Maclet. When P. Berry visited Tahiti, Michel Guérin and Jacques Florence provided kind hospitality and logistic support. In New Zealand, Phil Garnock-Jones and Peter and Elaine Johnson gave generous assistance to P. Berry. Lois Brako was a helpful field companion to P. Berry; both she and Colin Webb provided useful comments to improve the manuscript. Drawings, photographs, and maps were prepared by Eduardo Pérez (Unidad de Medios Audiovisuales, Universidad Simón Bolívar, Caracas, Venezuela), Ernst Beuzenberg, Pat Brooke, John Cocks, Robert Lamberts, Jim Miles (D.S.I.R., Christchurch, New Zealand), and Sabrina Malcolm (Landcare Research, Lincoln, New Zealand). We thank the curators of the following herbaria for allowing us to examine material under their care: AK, CHR, MO, OTA, PAP, WELT, and WELTU.
[2] Manaaki Whenua–Landcare Research, P.O. Box 69, Lincoln 8152, New Zealand.
[3] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, U.S.A.

F. excorticata (J. R. & G. Forst.) L. f. from New Zealand is found nowhere else in the genus. At the other extreme is the barely woody *F. procumbens* R. Cunningham ex A. Cunningham, a small-leaved and slender-stemmed, decumbent or scandent species that is confined to the far northern coasts of New Zealand. Between these in form is another New Zealand species, *F. perscandens* Cockayne & Allan, a woody liana when mature and found throughout most of the country. The fourth species, *F. cyrtandroides* J. W. Moore, is a small tree and occurs only on the island of Tahiti. All four species have blue pollen, in which they are unique in the genus, and they show a wide diversity in sexual systems, floral and fruit morphology, and seed anatomy.

The last account of the South Pacific species of *Fuchsia* was by Philip A. Munz in 1943. Since then much new information has become available, much of it unpublished, and has prompted a new evaluation of this far-flung outpost of a genus known throughout the world for its horticultural importance.

Systematic Background and History

The kotukutuku of the New Zealand Maori, now known as *Fuchsia excorticata*, was made known to science by Joseph Banks and Daniel Solander, botanists on Captain Cook's first voyage (1768–1771). They first collected it on October 20 and 21, 1769, at Tegadu (now Anaura Bay), which lies to the north of Gisborne on the east coast of the North Island of New Zealand. This was followed by collections at nearby Tolaga Bay, in the north of the North Island at Opuragi (now Mercury Bay), and in the Bay of Islands at Motuaro. It was also gathered at Totaranui in Queen Charlotte Sound in the north of the South Island. These localities are all noted in the unpublished manuscript entitled "Primitiae Florae Novae Zelandiae" (Beginnings of a Flora of New Zealand), which gives detailed descriptions of the plants collected by Banks and Solander. The original manuscript is kept at the Natural History Museum (BM), with a copy at the Landcare Research Herbarium at Lincoln (CHR). In this work, the kotukutuku was placed next to *Epilobium* in the Octandria Monogynia. Instead of recognizing it as a *Fuchsia*, Banks and Solander gave it the new genus name *Agapanthus* (from *agapylos*, pleasant, and *anthos*, flower) and named it *A. calyciflorus* in reference to the prominent calyx lobes. However, this name was never validly published. An attractive color plate showing a leafy shoot with flowers and fruits from Anaura Bay was published by Beaglehole (1962) in his edition of the "Endeavour" journal of Joseph Banks, and also by Begg & Begg (1970). The artist was James Miller and the painting was based on a preliminary sketch with notes on the flower color by Sydney Parkinson, one of Cook's artists who died during the return voyage. In the 1980s, color engravings from all the original plates prepared from Captain Cook's first voyage around the world were published for the first time as part of the *Banks' Florilegium* project at the Natural History Museum (BM), and *Fuchsia excorticata* was published as Plate 452 in Part XXII of the series (Banks, 1986).

The kotukutuku was also collected by John Reinhold Forster and his son George, botanists on Cook's second voyage (1772–1775). The locality was almost certainly Queen Charlotte Sound, either between 2 and 25 November 1773, or between 19 October and 10 November 1774, for these were the only times the botanists were on shore during the flowering season. Once again, this plant was not recognized as *Fuchsia*, and Forster & Forster (1776) named their plant *Skinnera excorticata*. The generic name was dedicated to a "percipient, far-seeing" botanist, the Reverend Richard Skinner (1729?–1795), M.A. Oxon, 1753, Rector of Bassingham, Lincolnshire, 1774 (Britten & Boulger, 1931). The specific epithet refers to the peeling bark. The Forsters gave clear drawings of the flower and fruit but curiously interpreted the little petals as nectaries and the berry as a capsule.

The first botanist to classify *Skinnera excorticata* as a *Fuchsia* was the younger Linnaeus (1781), who correctly identified the petals, but also interpreted the sepals and floral tube as part of the corolla. De Candolle (1828) made the first attempt at an infrageneric classification of *Fuchsia*, designating *Skinnera* as one of two sections in the genus; he based this decision on the alternate leaves, ventricose floral tube, and multiseriate ovules of *F. excorticata*.

Plants of the rare, creeping *F. procumbens* were first collected by Richard Cunningham in 1834 at Matauri Bay in northern North Island. He died in 1835 but his manuscript description was published by his brother Allan (Cunningham, 1839). In 1871 Joseph Hooker described a new species, *F. kirkii*, based on plants with larger flowers than those originally described for *F. procumbens*. Just a few years later, however, Hooker realized that the floral size differences were due to sexual dimorphism in the same species, with *F. kirkii* based on the larger male morph and *F. procumbens* based on the smaller female morph (Hooker, 1874).

While preparing his *Handbook of the New Zealand Flora*, Hooker recognized the presence of

fuchsias in New Zealand intermediate in size and habit between *F. excorticata* and *F. procumbens.* He published the species *F. colensoi* for a group of specimens quite variable in leaf size (Hooker, 1867). From one extreme of this variable complex, Cockayne & Allan (1927) described a new lianoid species, *F. perscandens,* from forest remnants near Feilding in the North Island. Their detailed field studies showed it to be distinct from the more variable and shrubby forms attributed to *F. colensoi* by Hooker (1867), Kirk (1899), and Cheeseman (1925). They wrote that the specific epithet indicated "a far more lianoid form than is seen in *F. colensoi.*" Thus "perscandens" was not intended to mean "climbing through," as claimed by Moore & Irwin (1978), but "very climbing," in agreement with the usage given by Jackson (1928) that in Latin compounds the prefix "per" increases their force. Allan (1927) also indicated that *F. colensoi* was based on some of the hybrid forms they found to occur frequently between *F. excorticata* and *F. perscandens.*

The last species in this group to be published was *F. cyrtandroides,* described by Moore (1940) from a single individual collected in 1927 from the highest mountain on Tahiti. The specific name is derived from *Cyrtandra* (Gesneriaceae), which has similarly opposite, strongly bicolored leaves in sympatric species such as *C. tahitensis* Nadeaud.

In the only overall treatment of the South Pacific species of *Fuchsia,* Munz (1943) recognized six species, including *F. kirkii* and *F. colensoi,* and placed them all in section *Skinnera.* He was obviously unaware of the sexual dimorphisms present in some of the species, as well as the variability of important morphological traits, for he incorrectly characterized the whole section as having alternate leaves, pendulous flowers, reflexed sepals, and strongly constricted floral tubes.

The present treatment recognizes four species of South Pacific *Fuchsia.* Three species remain in section *Skinnera,* also the common and variable hybrid entity, *F.* ×*colensoi.* However, a new section, *Procumbentes,* is proposed herein for *F. procumbens,* based on a series of fundamental differences from the other South Pacific species.

Habit and Deciduousness

The first resident botanist in New Zealand to describe the adult plant of *Fuchsia excorticata* was Thomas Kirk in his classical works, *The Forest Flora of New Zealand* (1889) and the unfinished *Students' Flora of New Zealand* (1899). In the former he wrote, "It is of irregular growth, varying from a much-branched shrub to a small tree 45 ft high, with a short trunk often gnarled, from 1 ft to 3 ft in diameter, and thin bark of a bright reddish-brown hue, which on old specimens divides into numerous paper-like flakes of layers."

This maximum height of *F. excorticata* was reported as 40 ft. by Cheeseman (1906, 1925), later converted to 12 m by Cockayne (1921, 1928) and Allan (1961). A tree of this size is shown in Figure 1, but such veterans are rare. It is now difficult to find trees taller than 9 m, at which height the crowns can be small in relation to the trunk and are often enveloped by lianas. When in the forest, the trunk can be single and well developed (Fig. 2), and is either erect or can slope toward the light above a stream, or even lie prostrate for 3–4 m until the streamside is reached, where it grows upwards. In more open situations, such as induced shrubland or along roadsides, the main trunk is less than 1 m tall and divides into several secondary trunks to form a large shrub up to 4 m high. Branching is erect (fastigiate; Kirk, 1899), and the growth of twigs is sympodial. Bussell (1968a) stated that the tips of *F. excorticata* shoots "shrivel and abort" at the end of the growing season and that growth is continued by the most distal remaining lateral bud. Christensen (1923) noted that branches of *F. excorticata,* partially buried by shingle rocks on the bed of the Perceval River near Hanmer, "had put forth numerous roots from 1 ft to 3 ft in length."

The wood anatomy of *F. excorticata* has been described by Meylan & Butterfield (1978) and Carlquist (1977). It can be considered quite mesomorphic, although the inner wood has shorter, narrower, and denser vessel elements than the outer wood. Growth rings are not evident in either the inner or outer wood, and starch is stored in the libriform fibers (Butterfield & Meylan, 1973). The wood is suitable for small cabinetry items and can be worked on a lathe to produce fine bowls and similar items. Contrary to Carlquist's report, crystals are present in the wood (R. Patel, pers. comm.). Twigs snap easily, but Kirk (1889) noted that "the wood is extremely difficult of combustion, and like the rewarewa (*Knightia excelsa* R. Br.) is often termed 'bucket of water wood' by the bushmen; even when thoroughly dry it can scarcely be burned in an open grate." The periderm in *Fuchsia excorticata* arises beneath the phloem sclerenchyma, and there is an extensive phelloderm layer (Robinson & Grigor, 1963).

Fuchsia excorticata stands out as one of the few deciduous trees indigenous to New Zealand. Russell (1936) described the mechanism of leaf abscission and found that leaf fall in Dunedin (46°S)

FIGURES 1, 2.—1 (left). A veteran tree of *Fuchsia excorticata* at Hinewai, Banks Peninsula, South Island, New Zealand. Overall height ca. 12 m; height to first branches 2.25 m; circumference (breast height) 2.5 m. Photograph by M. Oelderink.—2 (right). A leafless tree of *Fuchsia excorticata*, shortly before leafing out. Height of main trunk is 1.8 m, diameter at breast height is 28 cm. Kaituna Valley, Banks Peninsula, South Island, New Zealand, 6 September 1988. Photograph by E. J. Godley.

began during early May in 1934 and was complete within 3 to 4 weeks. The trees remained leafless during most of June, all of July, and early August, with new leaves appearing by the middle of August. Bussell (1968a, b) reported that leaf fall in Dunedin occurred throughout the growing season, with two distinct peaks, one in midsummer and the other in autumn. Bud break on marked shoots of *F. excorticata* began about 1 September in 1963 and on 20 August in 1964 (mean August and September temperatures in Dunedin were 1.5°C lower in 1963). This and other evidence led to the conclusion that the start of the growing season is controlled mainly by temperature. On the Port Hills near Christchurch (43°32′S), Suckling (1914) noted that leaves "are lost in May and appear in August."

In the North Island, the Auckland botanist Crookes (1926) stated that *F. excorticata* is "only deciduous in *mountain* districts of the North, but is regularly deciduous in the South" [italics ours]. Moore & Irwin (1978) stated that the leaves last through winter in the north. However, A. E. Esler (pers. comm., 25 Aug. 1980) noted a mixed pattern of leaf fall in local populations in Auckland (36°52′S) during the relatively mild winter of 1980—all mature bushes were completely deciduous, although young growth on stems up to 2 cm diameter and juvenile plants perhaps 2 to 3 years old retained their leaves.

In *Fuchsia cyrtandroides*, mature individuals range from strongly branched shrubs 2–3 m tall in exposed situations (Fig. 3) to small trees 5–6 m tall with irregular trunks and a stem diameter of 20 cm in small, more protected valleys. The bark is brown and smooth on young stems, not obviously peeling off the trunk as in *F. excorticata*. Carlquist (1975, 1977) presented some data on wood anatomy of *F. cyrtandroides*, indicating it to be highly mesomorphic for the family, with few, wide vessel elements. He suggested some seasonality in flowering and leaf production in this species when he stated that he had "seen *F. cyrtandroides* on Tahiti during July, the dry season, flowering on branches that are virtually leafless" (Carlquist, 1975). All trees observed in September 1973 by E. Godley were in full leaf and flower, and trees observed in February 1988 by P. Berry were all in full leaf, but flowers were extremely scarce.

FIGURE 3. Shrubby habit of *Fuchsia cyrtandroides* on an exposed ridge, Mont Marau, Tahiti. Photograph by P. Berry.

The adult of what is now called *Fuchsia perscandens* was described by Cockayne (1912) as "a shrub in the open and at times a scrambling liane in the forest." Bird (1916) traced the development of these growth forms (under the name *F. colensoi*) in Riccarton Bush, Christchurch. The primary shoot generally grows up to 35 cm high without support and then follows one of three pathways. It can push up between the branches of a shrub and search tor further support on emergence, or it can trail along the ground for 1–2 m, rooting and giving off lateral branches, one or more of which may find support. If no support is found, the stems may form prostrate or cushion-like masses up to 1 m high on the forest floor.

Shrubs in the open can apparently originate either from nonsupported plants that developed within a forest which was later opened up, or from plants which developed outside forest where no support was available. In exposed situations, the search for support is greatly impeded because shoots that protrude from the surface of the shrub are usually killed back by cold or dessication, and growth arises from lateral buds further back, as in *F. excorticata.* The extent and size of the lianoid form can best be seen in forest remnants where the support has collapsed and the cable-like stems, up to 6–7 cm in diameter, lie near the ground (Fig. 4). Plants cultivated without support usually adopt the open bushy habit of this species.

Bird (1916) noted that in Riccarton Bush, near Christchurch, "Plants in exposed situations lose their leaves early in winter, but in the shade they are devoid of leaves for only a few weeks." On roadside plants of *F. perscandens* in a bush remnant at the Sign of the Bellbird, Port Hills, Christchurch, E. Godley noted that on 5 May 1988 there were many dead leaves, perhaps due to a recent frost. On 10 June 1989, there were occasional leaves, but by 11 July all plants were leafless. In 1987, 1988, and 1989, leaves had appeared by the first week of October.

In contrast to Christchurch, a specimen of *F. perscandens* collected from the coastal lowlands at 39°56′S in North Island was in full leaf in July 1970 (Wanganui East, CHR 209534). Further inland and a little further south, specimens from several localities near Feilding showed new leaf flushes from early September to mid October.

Natural populations of *Fuchsia procumbens,* although confined to the warmer north of New Zealand, are nearly or completely deciduous in winter, when the bare slender stems are only made conspicuous by the red fruit. Even as early as 21 Jan-

FIGURE 4. Lianoid habit of *Fuchsia perscandens* in a forest remnant, North Canterbury, South Island, New Zealand. Diameter of the stem is 6.25 cm. Photograph by F. Allan.

uary, plants bare of leaf but full of berries have been recorded (Godley and Reynolds, pers. obs.). On the other hand, a plant cultivated at Akaroa, South Island (43°49′S), was evergreen. Thomson (1909) recorded that plants cultivated outdoors in Dunedin were killed during an exceptionally cold spell in July.

HABITAT

Fuchsia excorticata. This is the most widely distributed and common of the South Pacific species of *Fuchsia,* with a range from ca. 34°S near the North Cape of New Zealand to ca. 50°33′S on Auckland Island. Cockayne (1910) wrote, "There is hardly a forest in New Zealand where the native fuchsia, the kotukutuku of the Maoris, may not be seen." In his descriptions of New Zealand vegetation, Cockayne (1921) mentioned this species in North Island habitats as diverse as *Corynocarpus* coastal forest on the Wellington coast or Kapiti Island and *Libocedrus bidwillii* Hook. f.–*Podocarpus hallii* Kirk subalpine forest of Hauhangatahi (780–1140 m) and Mount Egmont (above 850 m). In South Island, the habitats include *Hebe elliptica* (Forster f.) Pennell coastal scrub at the base of Bluff Hill and *Podocarpus totara* D. Don forest on Banks Peninsula. Cockayne also mentioned *F. excorticata* as one of the principal species of small tree associations near riverbanks and as an invader after retreat of glaciers and after forest has been milled and burned.

At present, however, *F. excorticata* is quite uncommon in many areas, even though forest remnants or secondary shrubland are still present. Much research has shown that browsing by the introduced bushtail opossum (*Trichosurus vulpecula* Kerr) can play an important part in the death of *F. excorticata.* In 1946 and 1947 in the Orongorongo Valley, North Island, *F. excorticata* was the main opossum food in the lowland broadleaf-podocarp forest (Mason, 1958). Almost 30 years later, Fitzgerald (1976) noted that *Fuchsia excorticata* was no longer abundant anywhere in the 93-ha forest, and the few large plants remaining and their seedlings were constantly being browsed by opossums and other browsing animals. In the upper Waiho Valley, South Westland, South Island, Fitzgerald & Wardle (1979) noted serious damage to *F. excorticata* involving death or defoliation, whereas on the roadsides and forest margins in the lower part of the valley plants remained vigorous despite browsing. On Banks Peninsula, eastern South Island, *F. excorticata* was the plant food favored by the opossums (Gilmore, 1967). Despite this, *F. excorticata*

is still one of the most common roadside trees or shrubs on the peninsula, possibly because white clover and grasses are also important opossum foods in this predominantly pastoral area.

On Stewart Island, Wilson (1987) found the kotukutuku to be rather local but scattered throughout the island, having been reduced mainly by opossums. He noted that on certain inland sites, *Fuchsia* was probably never prominent, even before the introduction of browsing mammals, apparently due to low soil fertility.

A significant feature in the distribution of this species, considering the many-seeded edible fruit, is that it is poorly represented on the offshore and shelf islands. In the north, *F. excorticata* has not been recorded from Three Kings and Poor Knights Islands. It is found, however, on the Hen and Chicken Islands and on the two largest northern offshore islands, Little Barrier and Great Barrier. In the Bay of Plenty, it is not recorded from Mayor Island. In the south, it has been recorded only relatively recently on the two largest shelf islands. It was first gathered on the Chatham Islands in 1957 by Madden, who wrote about it, "Now spreading within the small area of Maipito Flats near Waitangi. Deliberately planted below the house many years ago by a nostalgic housewife" (Madden & Healy, 1959). The only collections since then have come from the same locality, Maipito to Lake Huro. In 1976, when visiting the island, G. Hamel (pers. comm.) was assured that *F. excorticata* was introduced there "about 90 years ago."

On the far southern Auckland Island, *F. excorticata* has also been found at only one locality. This population lies at the head of Laurie Harbour in the Ross Harbour area at the north of the island (ca. 50°33′S) and was found by B. C. Aston on 7–8 January 1909 (Cheeseman, 1909, appendix). Godley (1985) recorded that on 30 December 1962, he saw four trees near the mouth of the Grey Duck Stream, all with trunks 35–45 cm DBH. All were in flower and were hermaphroditic. These were the only trees seen while wading down the stream to its mouth, after descending to the valley from north of Cloudy Peak. In early April 1980, C. D. Meurk (1982) saw *F. excorticata* "scattered along a small tributary (from ca. 75 m elev.) entering south side of Grey Duck Creek ca. 200 m from the mouth, and down to sea level at the head of Laurie Harbour—in association with the rare *Asplenium bulbiferum* Forst. f." Just as the populations of *Olearia colensoi* Hook. f. var. *grandis* Simpson around Ross Harbour arose from a relatively recent introduction (Godley, 1965), so could this population of *F. excorticata*. But whereas the *Olearia* populations are definitely associated with the activity of man, *Fuchsia* apparently is not.

Fuchsia cyrtandroides. The interior of the island of Tahiti (ca. 17°32′S) is mountainous with an estimated rainfall of 3000 mm for Mt. Marau, and 5000 mm for Mt. Orofena (J. Florence, pers. comm.). Precipitous ridges lead to the central peaks, and tracks follow the ridges. It is along these tracks that *F. cyrtandroides* has mainly been found, although not in abundance (Fig. 5). Godley (1983) reported finding 11 trees on the Marau ridge at about 1400 m, and three trees high up the Aorai trail; he also noted that in the more temperate climate of these higher altitudes, the fuchsia grows in association with several other genera found in New Zealand, e.g., on Aorai: *Astelia, Blechnum, Cyathodes, Freycinetia, Gleichenia, Metrosideros,* and *Weinmannia.* Associated taxa observed by Berry in 1988 on the Marau ridge were *Cyathea* spp., *Cyrtandra tahitensis* Nadeaud, *Coprosma, Freycinetia, Metrosideros, Myrsine, Scaevola tahitensis* Carlquist, *Sclerotheca jayorum* J. Raynal, *Streblus, Vaccinium,* and *Weinmannia parviflora* Forst. f.

As for habitats other than ridge tops, M. van Balgooy, who visited Tahiti in September 1971, reported in a letter to P. Raven that *F. cyrtandroides* is much more common in the deep shaded valleys than along the ridges, where most of the paths are located. He considered it to be one of most common members of the montane forest between 1400 and 2000 m. In a letter to P. Raven in August 1969, F. R. Fosberg recalled that his 1934 collection of *F. cyrtandroides* was in one of the wettest, mossiest forests he had ever seen, with abundant tree ferns and fronds of *Asplenium nidus* L. up to eight feet long.

In 1983, a cyclone toppled and severely altered the tall cloud forest, which is now much more open; in February 1988, all trees seen by P. Berry on the Marau ridge were heavily affected by gall midges (Cecidomyiidae), whose larvae cause galls on leaves.

Fuchsia perscandens. The distribution given by Allan (1961) from 42°S in South Island to 40°14′S in North Island (forest remnant near Feilding) should be extended to 37°18′ to 46°36′S (Fig. 6). The northernmost record (as *F. colensoi*) was made by Cheeseman in January 1875, from somewhere in the Northern Wairoa (AK). All that we know is that during this "hurried examination" of the district, Cheeseman collected *Juncus tenuis* Willd. in the upper reaches of the Northern Wairoa River near Omana (35°54′S) and that he went higher up and lower down the river (Cheeseman, 1879). He

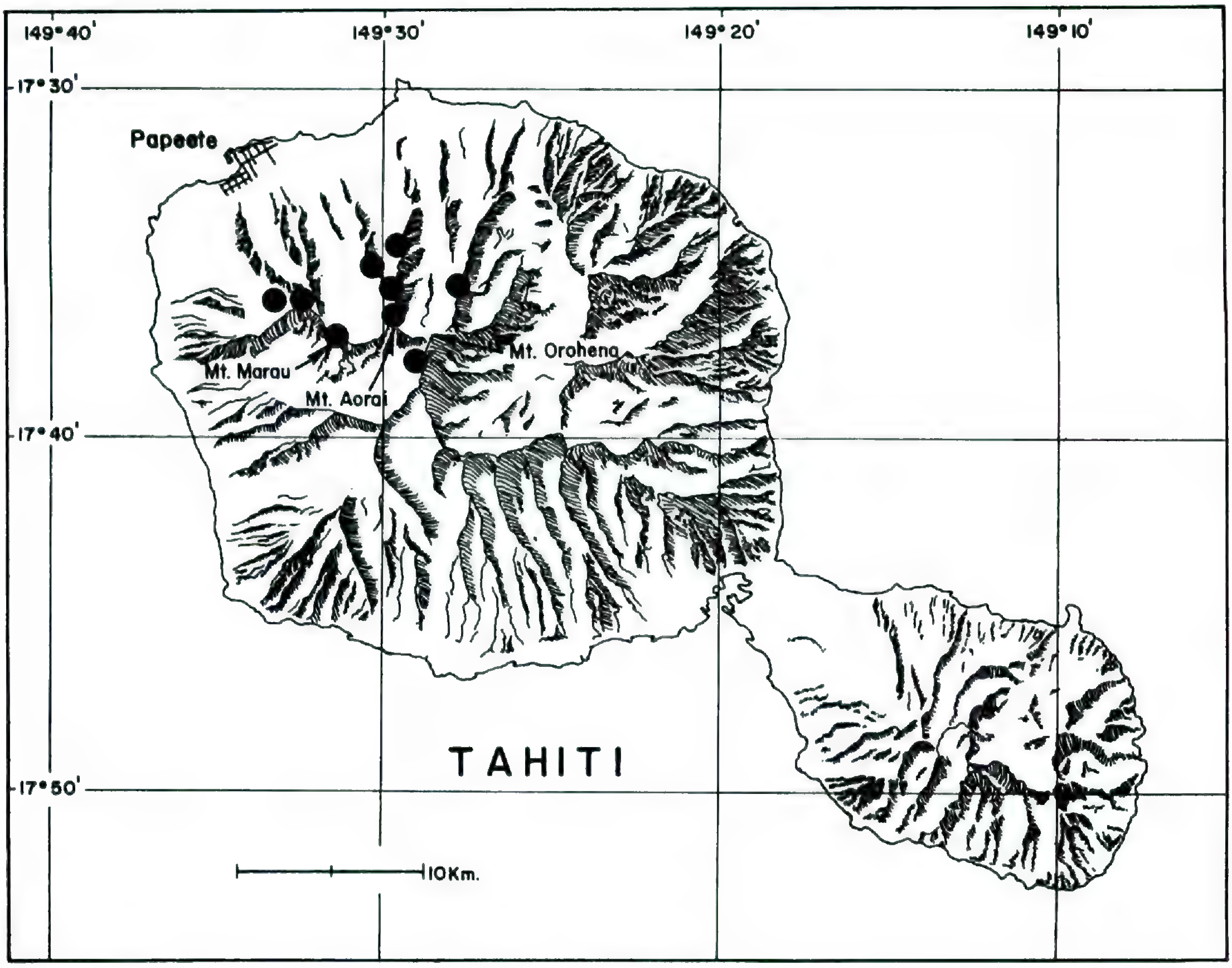


FIGURE 5. Native distribution of *Fuchsia cyrtandroides*. Drawing by E. Pérez.

may have approached the district from Whangarei. This is the only record of *F. perscandens* north of Auckland, and the population is probably now extinct due to forest clearing.

From just south of Auckland, in the lower Waikato, there are two other early records. In 1870 Kirk gathered specimens (WELT) from forest adjacent to Lake Whangape (37°28′S), noting, "A *Fuchsia*, of sub-scandent rambling habit, was found here, but without flower or fruit; it is perhaps a form of *F. colensoi*" (Kirk, 1871); and at nearby Opuatia Creek he found a "*Fuchsia* sp. in habit resembling *Muehlenbeckia adpressa*, no flower or fruit" (WELT). Both collections are *F. perscandens*, and their populations are probably extinct.

The northernmost localities known at present are at 37°15′S in swamp forest of *Dacrycarpus dacrydioides* (A. Rich.) de Laubenf. (white pine) and *Laurelia novaezelandiae* A. Cunn. near the Kopuku Coal Mine, where the species was locally common around tree bases in 1980 (CHR), and to the east at 37°18′S in derelict *D. dacrydioides* forest 12 km NW of Paeroa, where very few plants were left in 1980 (CHR). These are "relict populations of a probably abundant plant of the once very extensive lowland white pine forests in the lower Waikato area" (Bartlett, 1984).

At Te Aroha, in the Thames Valley, 20 km south of Paeroa, Cheeseman collected *F. perscandens* in 1884 (AK). But south of the lower Waikato and the Thames Valley, in the middle Waikato basin, *F. perscandens* continues to be rare. It was not listed by M. C. Gudex in his several reports on forest remnants, and *F.* ×*colensoi* was reported as rare (Gudex, 1963). In 1991, a single female plant was found in Hammond Bush, Hamilton (37°38′30″S) (CHR). The species is not recorded again until near Te Koraha (38°13′S) west of Otorohanga (AK). Further to the east, *F. perscandens* almost reaches the coast at Whakatane (37°58′S), Bay of Plenty (AK), again in a remnant of white pine forest; and in the far east of North Island it reaches ca. 38°33′S, 16 km north of Gisborne, where it was sprawling down a grassy bank (Cresswell, 1976).

Southwards, *F. perscandens* is found throughout both North and South Islands but is not common.

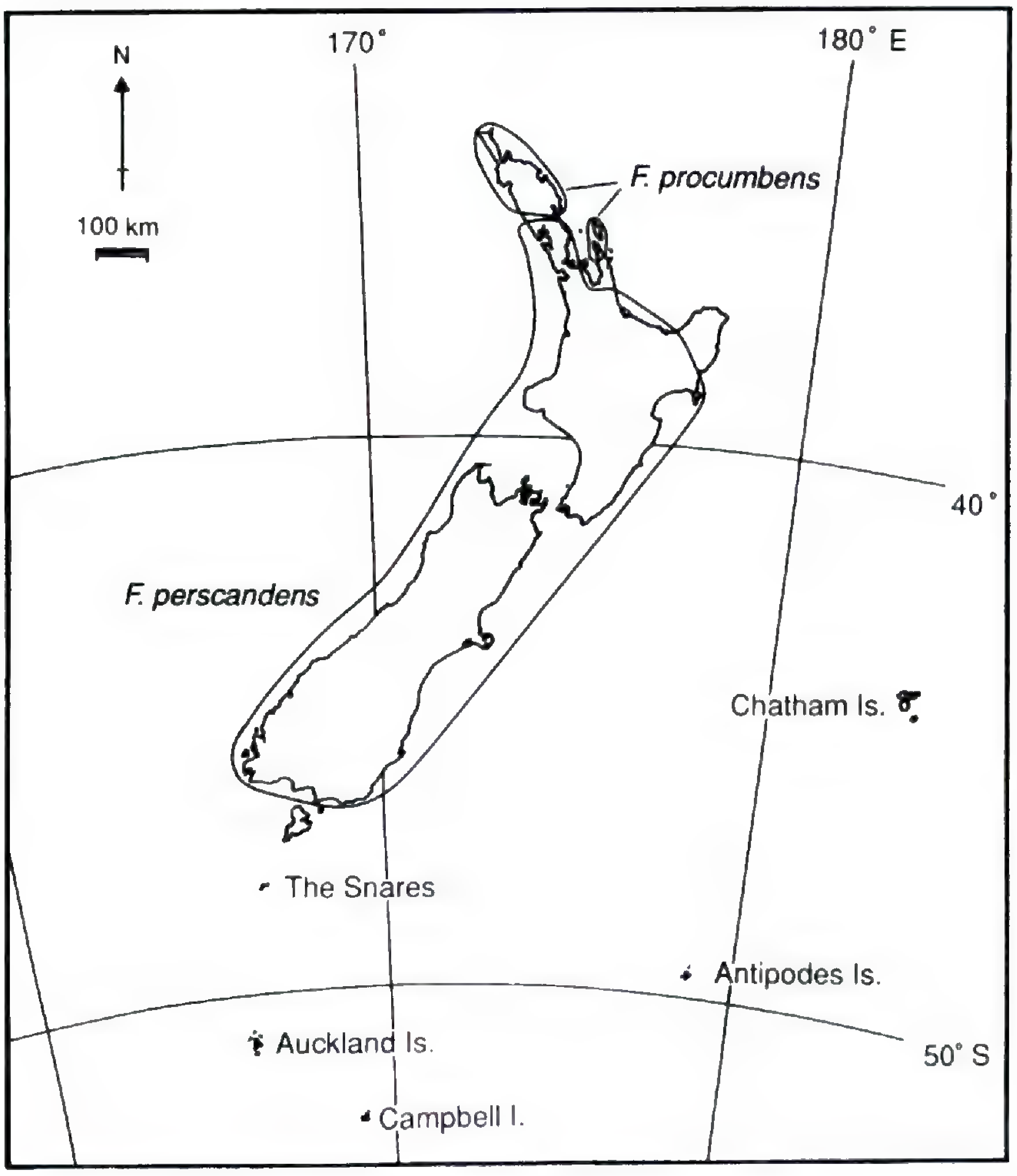


FIGURE 6. Native distribution of *Fuchsia perscandens* and *F. procumbens*, in New Zealand. There is currently no overlap in the distribution of these two species, with *F. procumbens* restricted to the northern part of the North Island. *Fuchsia excorticata* is widespread in New Zealand, occurring throughout the North and South Islands, as well as on Stewart Island (shown between The Snares and the southern tip of the South Island on the map), Chatham Island, and Auckland Island. Drawing by S. Malcolm.

It is recorded from coastal rocks and wind-shorn coastal scrub, in Westland, South Island; the highest altitudes are 1700–1800 ft. (518–549 m) north of Taihape in central North Island and 1500 ft. (457 m) on the Hunters Hills, eastern South Island. Common habitats are river terraces and remnants of white pine swamp forest, but it is also recorded from limestone in the Wairarapa (North Island) and Marlborough (South Island). The southernmost record is from coastal scrub at Bluff (46°36′S) on the shores of Foveaux Strait.

Fuchsia procumbens. This species is confined to the coastal regions of northern New Zealand in lower latitudes than *F. perscandens* (Fig. 6). The northernmost record is from Kerr Point (34°24′S) near North Cape. Southwards, *F. procumbens* reaches Maunganui Bluff (35°46′S) on the west coast of North Auckland, and Bream Head (35°51′S) on the east. It then reappears further east on Great Barrier Island, from where it extends south to Kennedy Bay (36°41′S) on the Coromandel Peninsula. It is not recorded from any of the many islands around the North Auckland and Coromandel coasts, except the largest, Great Barrier.

Since its discovery in 1834, *F. procumbens* has been recorded from 51 localities (38 in North Auckland; 6 on Great Barrier; 7 on Coromandel), and at several of these there is more than one population. From 1834 to 1901, only 15 localities were recorded, and Cheeseman (1906) understandably described *F. procumbens* as "rare and local." This impression was reinforced when only five new localities were added from 1907 to 1945. But since 1946, 31 more localities have been discovered, several the result of boat surveys of coasts otherwise difficult to reach (Godley and Reynolds, unpublished manuscript).

Despite its limited latitudinal and altitudinal range, *F. procumbens* occupies a diversity of habitats. Although not a true psammophyte, it grew at Matauri Bay on "the sands immediately above the range of the tide" (Cunningham, 1839), and Cheeseman (1897) found *Ipomoea palmata* Forssk. and *F. procumbens* "plentiful along the beach" south of North Cape. It is likely that these were old

foredunes, relatively stable, and with damp places for the *Fuchsia*.

On the northeast coast of Great Barrier Island, Wright & Cameron (1985) noted *F. procumbens* in a unique low herb field at four stream mouths. The associated species included *Arthropodium candidum* Raoul and *Leptinella dioica* Hook. f., reaching their northernmost distribution here, with *Ophioglossum petiolatum* Hook. forming the only large, secure population of this fern known in New Zealand. On sheltered rocky or shingle foreshores, where sandy beaches do not intervene, or on the margins of mud flats, *F. procumbens* can grow to within 2–3 m of the high tide mark. Here it can scramble among the irregular bases of the trunks of the pohutukawa, *Metrosideros excelsa* Soland. ex Gaertn. (Myrtaceae), a common coastal tree of northern New Zealand, or form carpets in the open. Further back from the coast, *F. procumbens* can be common on the floor of pohutukawa forest (particularly where light has been let in), where it scrambles through the sward of the grass *Microlaena stipoides* (Labill.) R. Br. or patches of the aggressive adventives *Stenotaphrum secundatum* (Walter) Kuntze and *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyu grass). Most woody species do not provide a suitable support for *F. procumbens*. An exception is the small shrub *Coprosma rhamnoides* Cunn., through whose dense divaricating branches *F. procumbens* scrambles to nearly 1 m above the ground.

Fuchsia procumbens is also regularly associated with two other native species, the flax, *Phormium tenax* Forster & G. Forster, and a cutty grass, *Cyperus ustulatus* A. Rich. It scrambles through flax clumps on the margins of streamlets (often summer-dry) in pohutukawa forest, or on steep open slopes above beaches; and it scrambles through the cutty grass in damp dune hollows (see above) or on old, dry, well-drained shingle beaches, or in local swampy patches. In disturbed areas, remnants of flax can provide a refuge for *F. procumbens*.

The highest altitude recorded for *F. procumbens* is on the precipitous coasts of the Cape Brett Peninsula. Here, the land mass rises steeply from the shore, and *F. procumbens* is found from near sea level to ca. 150 m elevation in a variety of situations—dry forest floor, moist stream sides, full sun and deep shade, gravelly soil, and dry clay.

Except for such a major change as roadmaking, human activity has not had obvious deleterious effects on populations of *F. procumbens*. It can be found draped over old fences or twining through the introduced blackberry (*Rubus fruticosus* L.). Long-term observations show that if already established, it keeps pace with the growth of buffalo grass and kikuyu and is not overwhelmed. Cattle do not appear to eat *F. procumbens*. However, trampling can bare the ground, leaving remnant patches of *F. procumbens* round the bases of trees or under fallen trunks. In reserves where cattle have been removed, *F. procumbens* is now flourishing. In such circumstances it seems highly probable that re-expansion is due to the scrambling and rooting habit of *F. procumbens*. Certainly this habit is very successful in maintaining unisexual populations, for example, an isolated female population on the Coromandel coast, first observed in 1929, was still present in 1990.

Fuchsia procumbens can still be described as local, but it is not as rare as described by Cheeseman (1906, 1925), and is not endangered as listed by Given (1981). It is also often grown in gardens. Only a few examples of local extinction are known, and the population with the longest known history (Mine Bay, Great Barrier Island) has existed from at least 1867 to 1989. Although recorded from several reserves, protection is not necessarily guaranteed there, particularly if the reserve is for recreation and frequented for swimming and picnics. Rather, *F. procumbens* is best protected by growing in relatively inaccessible places, or, if accessible, by its inconspicuousness and its similarity to the common twiner, *Muehlenbeckia complexa* Meissn.

Flowering Times

Fuchsia excorticata. The flowering time of August to December (late winter to early summer) given by Cheeseman (1906, 1925) and copied by Allan (1961) covers the period when plants will almost certainly be in flower in the lowlands. But it is not unusual for flowering to begin in June to July (early to mid winter) and to extend into January (mid summer), as shown by the following field notes made by E. Godley. On 10 June 1981, two out of four marked trees in the Kaituna Valley, Banks Peninsula, had scattered flowers. On 15 June 1977 at Gillespies Beach, Westland, several trees had flower buds, flowers, and green fruits; on 29 June 1980, at the Sign of the Bellbird, Christchurch, all four marked trees had flower buds, and one had open flowers. Cameron (1991) observed that on 3 July 1991 in Kauri Park, Auckland, plants of *F. excorticata* had many flower buds and flowers ranging from young to mature or shed, and Jones (1990) observed many flower buds and flowers on 14 July 1990 in Titirangi, Auckland. On Banks Peninsula, E. Godley found some trees still in flower on a plateau above Pigeon Bay on 21

FIGURE 7. Ramiflory in *Fuchsia excorticata*. Photograph by R. Lamberts.

January 1989, at ca. 500 m elevation, but on 24 January of the same year, there were no flowers remaining on four marked trees in the Kaituna Valley.

Individual trees also have a long flowering period. The first flowers are produced on the older branches or on the trunk; they appear when the trees are leafless and mature in acropetal succession on leafless condensed shoots (Fig. 7). The second wave of flowers is borne on leafy shoots produced from the youngest twigs; the flowers are solitary in the axil of each leaf and mature from the base to the tip of the twig. Both axillary and ramiflorous flowering continue to the end of the flowering period. For an hermaphroditic tree grown from seed from Kaituna Valley and cultivated at Lincoln, outside Christchurch, the flowering period lasted 145 days in 1989, with the following sequence: ramiflory only from 23 July to 22 September (61 days), then both ramiflory and axillary flowers from 23 September to 4 December (84 days). In the 1992 to 1993 flowering season, the same plant flowered for 130 days, ramiflorous only from 31 August until 16 October (47 days), then ramiflorous and axillary together from 17 October to 8 January (83 days).

An unusual situation was noted on Banks Peninsula involving a tree near Duvauchelle on a shaded southern aspect, and another at Pigeon Bay 2 km away with a more sunny northern aspect. When first seen on 31 March 1991 (early autumn), both bore flower buds, flowers, and fruits; and these three stages were present at approximately monthly intervals (although in varying amounts) until 18 December, a period of 262 days. There was then a gap from January to March 1992, when flowering began again.

The few observations at higher altitudes show a much shorter flowering period, mainly due to a later start. At the Maruia Springs Hotel (650 m elevation), in the north-central part of the South Island, three marked trees (two female, one hermaphrodite) were observed by E. Godley in late 1981 to early 1982 as follows: on 14 October, leaf buds were just beginning to open, and flower buds were up to 5 mm long and very sparsely scattered over the branches. By 26 December, the plants were in full leaf, with occasional flower buds and flowers at all stages of development, with green fruits present. By 8 January, plants were in full leaf, but two of the three plants had just finished flowering, and one female still had scattered flowers. An estimate of the flowering period there would be from 1 November to 12 January (73 days).

Fuchsia cyrtandroides. There appears to be some flowering seasonality in this species, based on the few observations available, with flowering concentrated in the drier part of the year. On Tahiti in September 1971, M. van Balgooy saw hundreds of plants of *F. cyrtandroides*, "Nearly all abundantly fruiting and some still flowering. . . apparently the flowering season is around July–September" (pers. comm. to P. Raven). Sherwin Carlquist observed *F. cyrtandroides* in June 1962 and commented, "The plants were flowering on virtually bare branches, a habit that suggests marked seasonality in flowering" (pers. comm. to P. Raven). In September 1973, all 11 plants seen by E. Godley on Mt. Marau were in flower, as well as the three plants seen on the Aorai trail. Paul Berry visited Mt. Marau in early February 1988, and out of about 25 small trees that were checked, only one individual was still in flower. This same tree had a few fleshy berries, while only two other trees had fruits remaining on the tree, and these were completely dried out.

Fuchsia perscandens. Flowers are produced, usually singly, from either the bare stems, or from the short stems of the leafy shoots. Allan (1961) gives a flowering time of September to October, but flowering specimens in CHR range from 20 August at Makino, near Feilding, to 25 March at Boundary

Creek, Rangitata River. In between these dates, the greatest number of flowering individuals were collected in October (14 specimens) and November (7 specimens).

Fuchsia procumbens. Flowers are produced singly in the leaf axils. Allan's (1961) flowering time of December to February can be extended from October to April. A continuous female sward at Kennedy Bay, Coromandel Peninsula, (possibly a single plant) was in full flower on 13 October 1990 and still had a few flowers and flower buds on 16 February 1991. These 126 days suggest a flowering time of 140 to 160 days. April flowering is recorded from Matauri Bay and Whangaruru in North Auckland (Godley and Reynolds, unpublished obs.).

Flowers and Breeding Systems

Fuchsia excorticata. Thomson (1881) recognized "two very distinct forms of flowers" in this species. The larger form was hermaphrodite and protogynous, and the smaller female. However, Kirk (1889) considered the flowers trimorphic, "the differences depending chiefly on the relative length of the stamens and style"; he also noted that his long-styled flower was female, and that his mid- and short-styled flowers were hermaphroditic. Later, Kirk (1893) related the situation in *F. excorticata* to the heterostyled trimorphic species studied by Darwin (1877). However, he added in a footnote, "If it were not for the two forms of hermaphroditic flowers, the New Zealand fuchsias might be termed gynodioecious." Kirk's classification was widely accepted until Godley (1955) showed that the situation in *F. excorticata* had nothing to do with true heterostyly. The division of hermaphroditic plants into "short-" and "mid-styled" forms could not be upheld, particularly as both types could be found on the same tree. The species is in fact gynodioecious (Fig. 8), with hermaphrodite plants self-fertile. In 12 natural populations the percentage of female plants averaged 26.9% (total of 1394 plants, with a range of 4.1% to 40.4% females).

Fuchsia perscandens. Allan (1927) also used the terminology of Kirk, but this species is again gynodioecious, with flowers that are smaller copies of *F. excorticata* (Godley, 1955). Twenty-six flowering individuals grown at CHR comprised 19 hermaphrodites and 7 females.

Fuchsia cyrtandroides. This species is hermaphroditic. As in *F. excorticata* and *F. perscandens,* the relative lengths of stamens and style (and hence stylar protrusion) can vary on the same plant

FIGURE 8. Floral morphs of *Fuchsia excorticata*; left, female; right, hermaphrodite. Scale bar = 5 mm. Photograph by R. Lamberts.

(Fig. 9). However, unlike these two species, the floral tube is not dilated at the base.

Fuchsia procumbens. This species stands apart from the other three in that the flowers are erect, yellow-tubed (not dilated at the base), apetalous, with the two whorls of anthers at almost the same level, and with only one-third to one-tenth the number of ovules. Plants are either female, hermaphrodite-male, or male, a situation that was described by Godley (1955) as trioecious. However, Godley (1979) later suggested that a better classification would be into females, males, and inconstant males, with the species then being subdioecious.

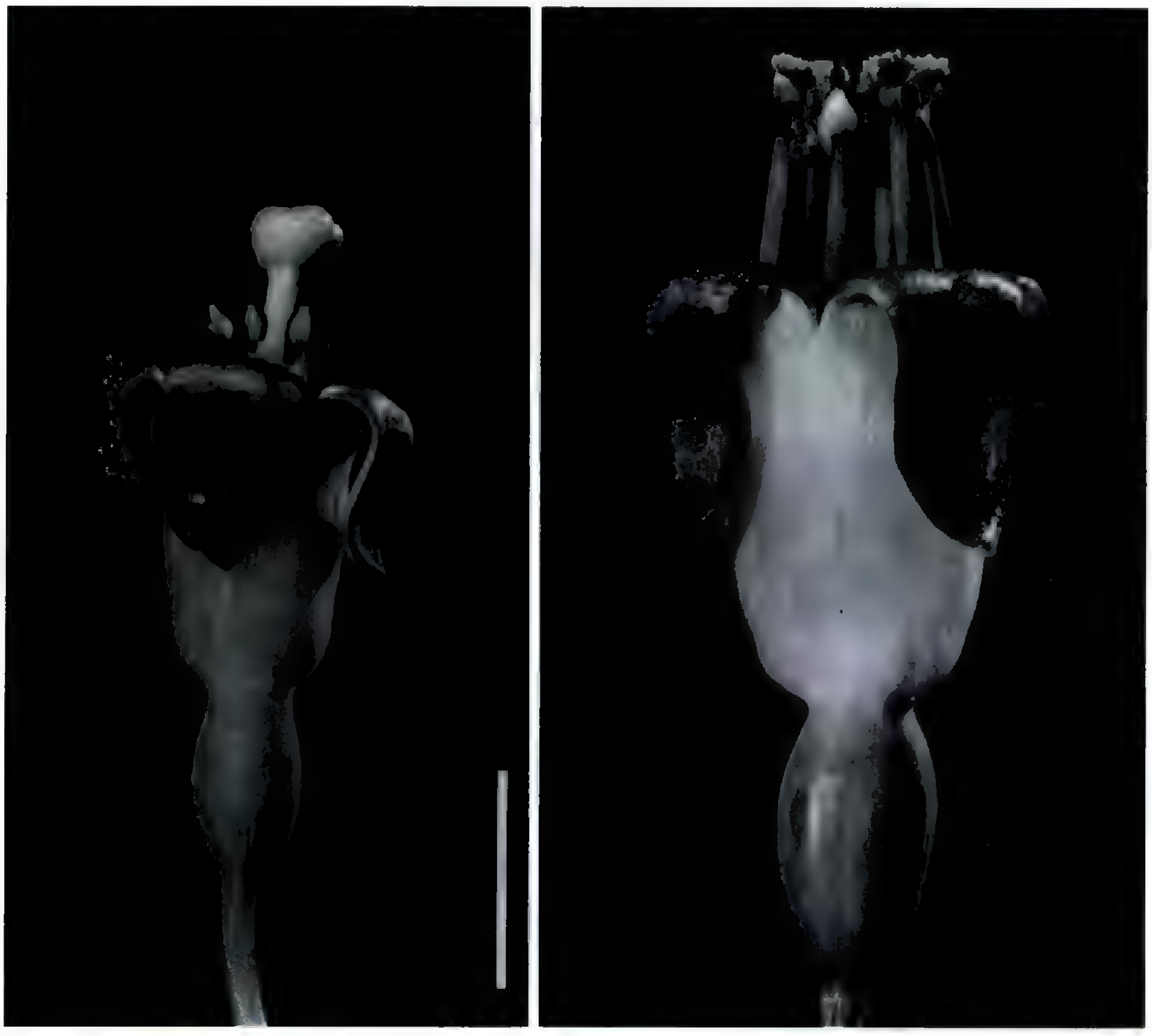
Female plants of *Fuchsia procumbens* are constant in their sex expression, with the female flowers approximately two-thirds the size of hermaphroditic and male flowers. Due to the tiny staminodes, the style is exserted with its large, globose stigma (Fig. 10); this led Kirk to classify the flower as "long-styled" (Kirk, 1893).

Plants of *Fuchsia procumbens* with only hermaphrodite flowers have never been found. When hermaphroditic flowers do occur, they are mixed with male flowers on hermaphrodite-male plants, but such plants are uncommon in nature. Hermaphroditic flowers are self-fertile, with the large, globose stigma situated at the same level as the anthers. They are also much less common than male flowers on the same plant. In the latter half

FIGURE 9. Flowers of *Fuchsia cyrtandroides*. Scale bar = 10 mm. Photograph by R. Lamberts.

of the 1950 to 1951 flowering season, from 5 December to 13 February, a single plant of *F. procumbens* produced 16 hermaphrodite flowers to 152 male flowers (data courtesy of S. D. Baker on a plant cultivated by J. M. Dingley in Auckland). Holdsworth (1959) showed that over a flowering season there is no recognizable trend in the proportion of the hermaphrodite and male flowers.

Maleness in *F. procumbens* is simply due to the lack of a stigma, and male flowers have ovules that are similar in size and number to those of females and hermaphrodites (Table 1). The style length var-



FIGURES 10, 11.—10 (left). Female flower of *Fuchsia procumbens*. Scale bar = 5 mm.—11 (right). Male flower of *Fuchsia procumbens*. Scale is the same as for Figure 10. The hermaphrodite flower differs only in having a well-developed stigma within the ring of anthers. Photographed by C. J. Miles.

Table 1. Ovule numbers in different sexual morphs of *Fuchsia procumbens* (5 flowers per plant).

Sex						Average	Voucher, source locality
Male	127	145	162	207[a]	230[a]	174.2	G2209, Matapouri
Male	90	102	109	131	188	124.0	G2208, Matapouri
Male	92[b]	125	133	141	155	129.2	G2207, Houhora
Male	76	99	121	125	128	109.8	G2201, Houhora
Male	75	81	90	138	140	104.8	G2204, Ngahau
Male	100	106	121	130	131	117.6	G1785, Whangaruru
Male	102	104	107	107	114	106.8	G1786, Whangaruru
Male	51[b]	65	80	82	105	76.6	G2202, Whangarei Heads
Female	80	88	96	97	109	94.0	G2211, Ngawae
Female	117	118	120	124	137	123.2	G1787, Whangaruru
Female	88	98	101	104	116	101.4	G2210, Coromandel
Hermaphrodite-male	76	99	121	125	128	109.8	G2205, Dingley (cultivated)

[a] Fruit with six locules.
[b] Fruit with three locules.

ies from the level of the anthers, as in hermaphroditic flowers, down to the length of the tube. Thus the "mid-" and "short-styled" flowers of Kirk (1893) can occur on the same plant. Male plants are the most common of the three kinds in the wild, with the style usually reaching no further than the mouth of the tube (Fig. 11; such plants were originally called *Fuchsia kirkii*). When Beuzenberg (1966) treated a constant male with gibberellic acid, a normal stigma developed, and a selfed progeny was obtained. In male flowers, the four bundles in the style remain unlignified, and this could be the cause or the result of the acephalic style.

Inheritance of male sterility. In the subdioecious *F. procumbens*, the female is heterogametic with male sterility controlled by a single dominant gene (Godley, 1963). That the same situation occurs in *F. excorticata* was shown by hand pollination of three trees growing in the Kaituna Valley, Banks Peninsula. When selfed, trees 1 and 2 (both hermaphrodites), produced 11 and 30 hermaphrodites, respectively, with no females. Crosses between tree 3 (female) and tree 1 produced 9 hermaphrodites and 12 females; tree 3 × tree 2 gave 60 hermaphrodites and 65 females. Progenies resulting from open pollination were also reared from each tree, with tree 1 producing 21 hermaphrodites, tree 2 giving 42 hermaphrodites, and tree 3 producing 23 hermaphrodites and 23 females.

A different genetic control of male sterility occurs in section *Encliandra* of Mexico and Central America. A statement by Breedlove (1969) shows that in *F. microphylla* HBK and *F. thymifolia* HBK it is the hermaphrodite that is the heterogametic sex, and also that the genetic basis of male sterility is not as simple as in the South Pacific fuchsias. Breedlove reported that selfed hermaphrodite plants gave progeny with "approximately 50%" of females. Even though lower proportions of females were obtained by Arroyo & Raven (1975) in *F. thymifolia* (42.9%) and *F. microphylla* (34.6%), when hermaphrodite plants were self-fertilized, these proportions are still much higher than the 25% expected if male sterility were controlled by a single recessive gene.

Floral pigments. In an extensive study of flower color pigments in *Fuchsia*, Crowden et al. (1977) found the 3-glucosides and 3,5-diglucosides of the six common anthocyanins to account for nearly all flower color in the genus. However, no detectable levels of the orange-producing pigment pelargonidin were found in flowers of *F. excorticata* and *F. procumbens*, which were characterized mainly by a combination of malvidin, peonidin, and petunidin. The characteristic purple anthers of *F. excorticata* were rich in petudinin, malvidin, and cyanidin. Preliminary analysis of the blue pollen of *F. excorticata* shows it to be a bound anthocyanin, probably forming a blue metallo-flavone-anthocyanin complex in living flowers (N. H. Fischer, pers. comm. to P. Raven in September 1988).

Pollen. *Fuchsia* pollen has been well studied by Nowicke et al. (1984), Praglowski et al. (1983), and Skvarla et al. (1976). The New Zealand species were also treated by Moar (1993). Besides having the unique suite of onagraceous pollen features such as viscin threads, protruding apertures, paracrystalline-spongy ektexine, and solid endexine, *Fuchsia* pollen is distinctive in its predominantly 2-aperturate grains with wide and short colpi or pores. Furthermore, the grains are always shed sin-

gly. Within the genus, the South Pacific fuchsias are generalized in features such as the 2-aperturate grains, segmented-beaded viscin threads, and globular type of ektexine sculpture. While the pollen of the South Pacific species of *Fuchsia* is considerably smaller than the American species (Praglowski et al., 1983), and some of the American species have other distinguishing characters such as smooth viscin threads or 3-aperturate grains, it is not possible to distinguish between any of the South Pacific *Fuchsia* species by pollen alone (Daghlian et al., 1985).

Fossil *Fuchsia* pollen is known from New Zealand from as early as the late Oligocene, around 27 mya (Mildenhall, 1980), and then extends up to the Pliocene and possibly into the Quaternary (Pocknall & Mildenhall, 1984). Although the fossil grains have been assigned to *Diporites aspis* Pocknall & Mildenhall, detailed ultrastructural analysis by Daghlian et al. (1985) has shown that they clearly represent *Fuchsia* pollen. Recently, fossil *Fuchsia* pollen dating back to the early Oligocene (at least 35 mya) has been documented from eastern Australia; these findings represent the oldest fossil records of the genus. The most recent records of fossil pollen in Australia, however, extend only to the middle Miocene, about 15 mya (Berry et al., 1990). Thus, it is likely that *Fuchsia* was once widespread at least in eastern Australia but went extinct there as the continent moved steadily northward and entered into increasingly more arid climatic zones.

Pollination

Fuchsia excorticata is well known to be pollinated by honeyeater birds (Meliphagidae) in New Zealand (Potts, 1871; Kirk, 1893; Thomson, 1927; Godley, 1979; Craig et al., 1981; Delph & Lively, 1985). The main visitors are the bellbird (*Anthornis melanura*) and the tui (*Prosthemadera novae-zelandiae*), although the rare stitchbird (*Pogonornis cincta*), which is now confined to Hen, Cuvier, and Little Barrier Islands (Bartle & Sagar, 1987), has been reported to visit *Fuchsia* in the north (Kirk, 1893). During the flowering season, blue *Fuchsia* pollen can conspicuously cover the birds' foreheads, which was first noted by Potts (1871) in bellbirds. Birds such as silvereyes (*Zosterops lateralis*), as well as bumblebees (*Bombus* spp.), also feed off the flowers of *F. excorticata*, but they rob the nectar without much effect upon pollination (Delph & Lively, 1985).

The flowers of *F.* ×*colensoi* and *F. perscandens* are similar to those of *F. excorticata*, but pollinators have not been recorded on them. In the shrub form of *F. perscandens* it would be difficult for birds to reach the flowers, which are usually produced inside the tangle of wiry branches.

On Tahiti no native birds have been observed on the flowers of *F. cyrtandroides*. However, silvereyes (*Zosterops lateralis*), which were first observed on the island (self-introduced?) in 1939 (Holyoak, 1974), were observed on *F. cyrtandroides* "flying from flower to flower, probably searching for nectar" in September 1971 (M. van Balgooy, pers. comm. to P. Raven). Since *F. cyrtandroides* is hermaphroditic, it is probably able to produce selfed seed as well. Meliphagid birds are not represented on the list of known extinct Tahitian birds by Holyoak (1974).

Despite close observation of native populations of *F. procumbens* by K. Reynolds, E. Godley, and T. Raven, no pollinator activity has been seen. None of the bird pollinators of *F. excorticata* has been seen visiting the flowers of *F. procumbens*, nor have any other birds. We should note, however, that the bellbird is extremely rare in the North Auckland sector where *F. procumbens* occurs. Thus Buller (1878) received first-hand reports from Whangarei that, "In 1859 this bird was very abundant, in 1860 it was less numerous, in 1862 it was extremely rare, and from 1863 to 1866 I never saw but one individual. It now seems to be entirely extinct in the district." With regard to the possibility that earlier bird pollinators of *F. procumbens* are now extinct, with the exception of subfossil remains of tui, bellbird, and stitchbird, there are no records of fossil Meliphagidae in New Zealand. Among other extinct birds, there are likewise none which could reasonably be suggested as possible pollinators (P. R. Millener, pers. comm.).

On the Poor Knights islands off the North Auckland coast, where bellbirds occur (Bartle & Sagar, 1987) but *Fuchsia procumbens* does not, geckoes in the genus *Hoplodactylus* regularly take nectar from three coastal plant species and are dusted with pollen (Whitaker, 1968, 1987). It has been suggested that lizards could pollinate *F. procumbens* on the mainland (E. K. Reynolds, pers. comm. to E. Godley, Dec. 1979 and to P. Raven, Oct. 1980; Whitaker, 1987). However, at Deep Water Cove, Cape Brett Peninsula, where skinks are plentiful (e.g., Lizard Bay), no pollinator activity on *F. procumbens* was observed by Mrs. Reynolds and F. J. Newhook, either during the day or throughout one night's observations. At Deep Water Cove, there was a large colony of small native bees near a population of *F. procumbens* (both male and female plants), but no floral visits were made, despite close observation by E. K. Reynolds, F. J. Newhook, and T. Raven.

Mrs. Reynolds has since caught a native bee with blue pollen on it, on a male plant of *F. procumbens* in her garden at Whangarei.

The flowers of all South Pacific species of *Fuchsia* are scentless and produce copious nectar at the base of the floral tubes. The nectar is high in hexoses and low in sucrose, which is consistent with other species pollinated by passerine (perching) birds in both the Old and New Worlds (Baker & Baker, 1979). The following ratios of sucrose to hexoses (combined glucose and fructose) in New Zealand fuchsias were obtained by Herbert and Irene Baker (unpublished data): 0.098 in *F. procumbens*, 0.059 in *F. excorticata*, and 0.033 in *F. ×colensoi*. The predominantly hummingbird-pollinated American species of *Fuchsia*, on the other hand, have sucrose-rich nectars with ratios ranging from 0.571 to 5.253.

Flower color. The flower color of *F. excorticata* was described by Kirk (1889) as "at first greenish, streaked or blotched with a dull deep purple, passing at length into a dull red." Delph & Lively (1985, 1989), who called the first phase green, were the first to study the development and significance of these changes. They found that open flowers of both sexes last an average of 11 days. About halfway through this period, they pass from green-purple to bright red over a transition period of 1 to 2 days. The change is age-dependent and not pollination-induced. Nectar is produced only during the initial green-purple phase, when pollination occurs. Delph & Lively (1989) suggested that red flowers, which are post-reproductive, act as a cue to direct pollinators away to the potentially reproductive green-purple flowers.

Flowers of both *Fuchsia perscandens* and *F. ×colensoi* exhibit the same color change pattern as *F. excorticata*. In 1988, P. Berry was able to confirm in Tahiti that native-growing plants of *Fuchsia cyrtandroides* also exhibit two different color phases; its flowers change during anthesis from a dull green with purple streaks to a uniform deep purple. Only *F. procumbens* differs fundamentally from the other South Pacific species. It passes gradually from a uniform green in bud to a yellowish floral tube during anthesis; the sepal lobes reflex back completely against the tube and turn a contrasting dull purple. Thus, there appears to be no clear separation in this species between the reproductive and post-reproductive phases of the flower. This may be due to a longer period of anthesis in *F. procumbens* than in the other species in the section, and detailed observations would be welcome on the length of anthesis and progression of color change in this species.

FIGURE 12. Berry of *Fuchsia procumbens*. Photograph by R. Lamberts.

Fruits and Dispersal

As with the flowers, the fruit of *Fuchsia procumbens* is distinct from that of the other three South Pacific species. This species produces a hollow, red, elliptic berry, up to 2.5 × 1.6 cm, with a fleshy pericarp 2–3 mm thick and thinner septa (Figs. 12, 13). At the inner angle of each of the four hollow locules there are two rows of seeds attached to the central fleshy axis, which is ca. 1 mm in diameter. Seed numbers in nine fruits resulting from natural self-pollination of an hermaphrodite-male plant with ovule numbers from 76 to 128 (see Table 1) were: 17, 29, 32, 42, 44, 44, 59, and 73. In a later year six fruits gave 28, 40, 59, 60, 66, and 69 seeds. Parthenocarpic fruits, smaller than normal, are produced when pollen is applied to the acephalic style of the male plant (Godley, 1955, 1979) or in isolated female populations (Godley and Reynolds, unpublished manuscript).

The fruits of the other South Pacific species are smaller, more fleshy, more uniformly globoid or oblong, purple when ripe instead of red, and with multiseriate ovules embedded in pulp in each locule. They have about 10 to 20 times the number of seeds as *F. procumbens*, and the seeds are much smaller. Parthenocarpy has not been observed in

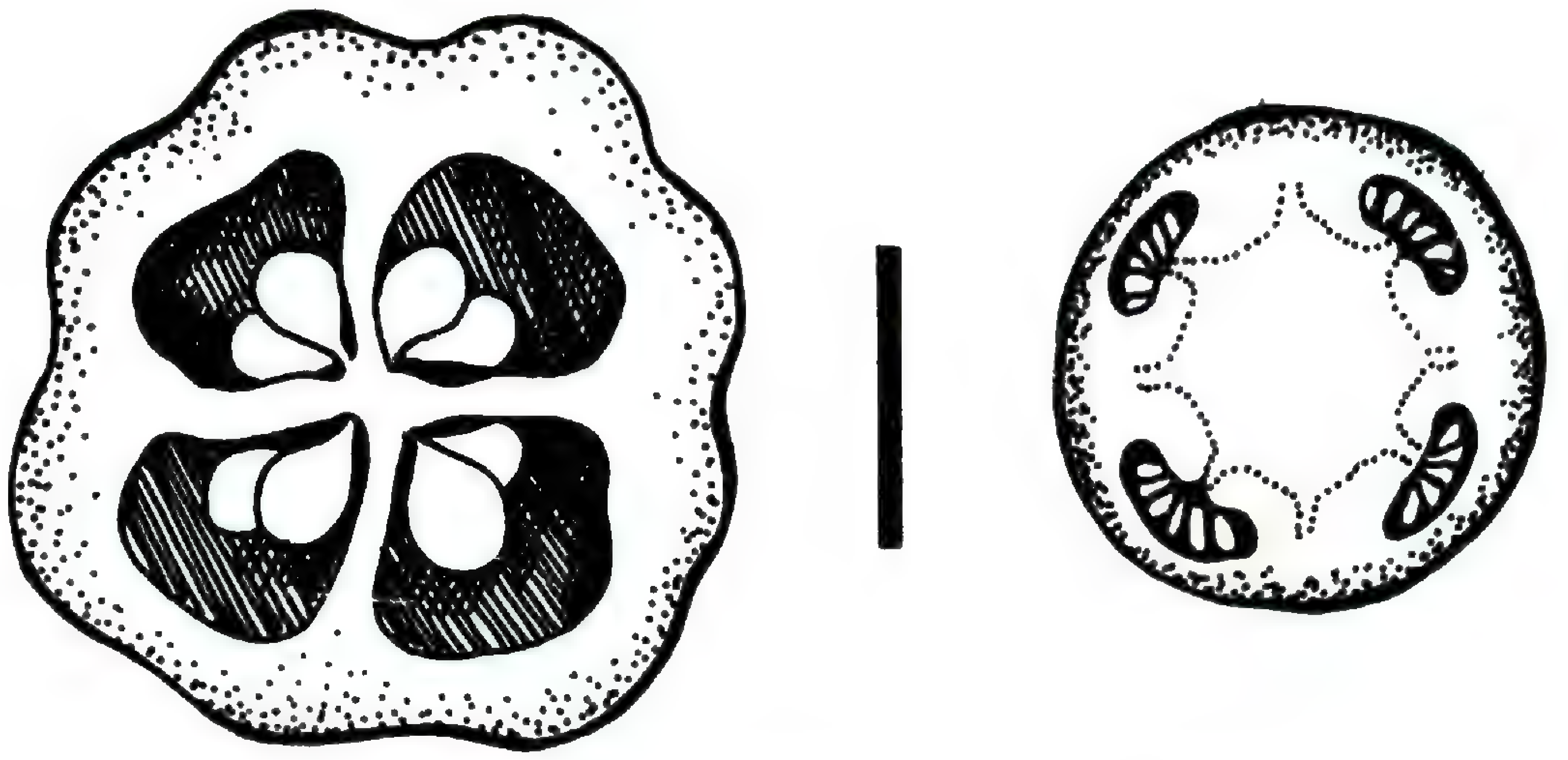


FIGURE 13. Cross sections of fruits of *Fuchsia procumbens* (left) and *F. excorticata* (right). Scale bar = 5 mm. Drawing by P. A. Brooke.

any of the other South Pacific species. The berry of *F. excorticata* is oblong, purplish black, and up to 1.5 × 0.7 cm. It is fully fleshy, with the pericarp, septa, and placentae filling the fruit, except for the four peripheral groups of very small imbedded seeds (Figs. 13, 14). Seed numbers and germination rates six weeks after sowing are given in Table 2. The overall germination after six weeks was 62.2%. The berry of *F. perscandens* is a smaller version of *F. excorticata*, while *F. cyrtandroides* can be distinguished by the shape of the apex and the tapering base (Fig. 15). Two fruits of the latter derived from hand-pollinations measured 1.5 × 1.0 cm with 568 seeds (from selfing) and 1.0 × 0.7 cm with 576 seeds (from crossing).

Dispersal. Berries of *Fuchsia excorticata* (konini, in Maori) are eaten freely and dispersed by the native New Zealand pigeon (*Hemiphaga novae-zelandiae*; Cockayne, 1928; McEwan, 1978) and other birds. On Kapiti Island tuis eat the konini, while bellbirds do not even wait until the berries are ripe, but eat them as soon as they show color (Wilkinson, 1952). In Dunedin, Thomson (1901) noted that *F. excorticata* became more abundant in the Town Belt with the increase of introduced blackbirds (*Turdus merula*) and thrushes (*Turdus philomelos*). These same birds are likely to eat and disperse the very similar berries of *F. perscandens* and *F.* ×*colensoi*. *Fuchsia cyrtandroides* also has juicy, many-seeded berries, but their possible dispersal agents in Tahiti are unknown and may have included birds that were driven extinct after human colonization. Dur-

FIGURES 14, 15. Berries of *Fuchsia excorticata* (left two, Fig. 14) and *Fuchsia cyrtandroides* (right two, Fig. 15). Scale bar is divided in 1 mm units. Photographs by R. Lamberts.

Table 2. Seed numbers and germination rates in fruits of *Fuchsia excorticata*.

	Average seed number per fruit	Range	Average germination percentage	Number of fruits
Plant 1 (hermaphrodite) selfed	475	324–552	67.5	9
Plant 2 (hermaphrodite) selfed	662		47.3	1
Plant 2 × plant 1	602	563–641	65.2	5
Plant 3 (female) × plant 1	489	398–550	67.0	5
Plant 3 × plant 2	539	395–614	56.0	10

ing a visit by P. Berry to native populations of *F. cyrtandroides* in February 1988, a number of trees with mature fruits were seen, but there were no signs of fruit removal by birds or other animals. Some berries were found still attached to the branches, but they were completely dried out and split open to expose the tiny seeds. *Fuchsia procumbens* is unusual in its larger, more leathery, fewer-seeded, and longer-lasting fruits, but nothing is known about its dispersal mechanism.

Seed Morphology and Anatomy[4]

To assess differences in seed morphology and anatomy in the South Pacific species of *Fuchsia*, the following specimens were examined: *Fuchsia cyrtandroides* (*Fosberg 61078*, US), *F. excorticata* (*Walker 4317*, MO), *F. perscandens* (*Raven & Simpson 25567*, MO), and *F. procumbens* (*Engelhorn 25719*, MO). Overall seed shape and seed surface morphology were observed using a Hitachi S2050 scanning electron microscope at 10 and 15kV. Dry seeds were soaked once in water and dehydrated through an ethanol series, critical-point dried in CO_2, and coated with platinum. For anatomical study, dry seeds were soaked once in FAA (5:5:90, using 50% ethanol), dehydrated through a t-butyl alcohol series, and embedded in Paraplast with a melting point of 56–58°C. The seeds were sectioned with a rotary microtome following standard paraffin methods. Sections cut at 8–10μ thickness were stained with Heidenhain's hematoxylin, safranin, and fastgreen FCF, and mounted with Entellan.

In all four species studied, mature seeds are slightly curved and ellipsoid or broadly ovoid with a small raphe. Seed sizes, based on measurements of five seeds in each species, are as follows: 1.10–1.14 mm long and 0.52–0.64 mm wide (between raphe and antiraphe) in *Fuchsia cyrtandroides*, 0.77–0.88 mm long and 0.39–0.46 mm wide in *F. excorticata*, 0.83–0.96 mm long and 0.52–0.63 mm wide in *F. perscandens*, and 2.39–2.43 mm long and 1.26–1.43 mm wide in *F. procumbens* (Figs. 16, 19, 22, and 25). Seed surface is scalariform in *Fuchsia cyrtandroides*, *F. excorticata*, and *F. perscandens*, but reticulate-foveate in *F. procumbens*.

The mature seeds of all four species are exalbuminous, as are all other species of *Fuchsia* and Onagraceae in general. The seed coat is typical of other members of the genus, composed of an exotesta, a multi-cell-layered mesotesta, and endotesta, a fibrous exotegmen, and an endotegmen (Figs. 17, 18, 20, 21, 23, 24, 26, 27, and 28). In all four species the cells of the exotesta are characteristically enlarged, while cells of the meso- and the endotesta are much smaller. The mesotesta is three- to five-cell-layered except in *Fuchsia excorticata*, where it is two- to three-cell-layered. The cells of the mesotesta are densely tanniferous except in *Fuchsia procumbens*. The cells of the endotesta are relatively clearly distinguished from the mesotestal cells in *Fuchsia cyrtandroides*, *F. excorticata*, and *F. perscandens* because they are thick-walled and contain crystals, but they are hardly distinguished from the mesotestal cells in *F. procumbens* because of the lack of such features.

Cells of the fibrous exotegmen are longitudinally elongate. They are narrow and about 2–5μ thick in *Fuchsia cyrtandroides*, *F. excorticata*, and *F. perscandens* (Figs. 18, 21, 24), but are extremely wide and about 30–40μ thick in *F. procumbens* (Figs. 27, 28). Cells of the endotegmen appear circumferentially elongate and thus criss-cross with longitudinally elongate exotegmic cells, and they are always tanniferous.

On the basis of seed morphology and anatomy, the four South Pacific species of *Fuchsia* are characterized by their enlarged exotestal cells, which is an apomorphic character not found anywhere else in the genus. Within this group, *Fuchsia procumbens* is clearly distinct from the others in seed size and seed surface sculpturing, as well as in the thickness of the fibrous exotegmen. *Fuchsia cyrtandroides*, *F. excorticata*, and *F. perscandens* can be

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distinguished from each other by seed size and thickness of the mesotesta, but they closely resemble each other in sharing small seeds with a tanniferous mesotesta.

Seedlings

In *Fuchsia excorticata,* the cotyledons are epigeal with a hypocotyl 5–7 mm long. They are glabrous, entire, without obvious veins, green on both surfaces, and reach a length of 3 mm. Their shape is roughly deltoid with a slight shoulder on the upper half of each lateral margin. Observations on 5 seedlings showed that pairs of opposite and decussate leaves are produced at the first few nodes, followed by subopposite pairs in which the leaves are offset vertically from 2 to 8 mm. The sequence of opposite and subopposite nodes was as follows: 2+5, 3+5, 4+4, 5+4. In the fifth seedling, however, the leaves were opposite at nodes 1–3 and 5–6, but subopposite at 4 and 7. From then on, the leaves are produced singly at each node and are evenly spaced with a phyllotaxis of approximately ⅖. The margins of the first leaves show small, spaced teeth not seen in the later leaves, while the undersurface of the first pair of leaves resembles that of the adult leaf in being silver-white tinged with pink. Tiny leaves begin to appear on very short shoots in the axils of the cotyledons after the first three pairs of leaves have been produced (about 50 days after germination), followed consecutively by buds in the axils of the upper leaves. Cotyledons of *F. perscandens* seen at Macdonald Downs, North Canterbury, in March 1988, were similar to *F. excorticata.*

In *Fuchsia cyrtandroides,* the cotyledons are similar in size and shape to those of *F. excorticata* but bear scattered hairs. In contrast to *F. excorticata,* the seedling leaves have shorter petioles, are more elliptic, and are always opposite and decussate.

Despite its small adult size, *Fuchsia procumbens* has the largest seeds and cotyledons of the native South Pacific fuchsias. Its cotyledons differ from *F. excorticata* in having a more flattened stalk, reaching 5 mm long, and a blade that is 5 mm long and 5 mm at the widest point. On a 2-month-old seedling, the main stem was 25 mm long, the hypocotyl 10 mm long and almost 1 mm thick, and the main root 45 mm long with abundant development of secondary roots, giving a root system 9 cm long when extended. The pair of cotyledons and the pairs of leaves at the four subsequent nodes were opposite and decussate; lateral shoots had developed in the axils of the cotyledons and the first two pairs of leaves. These lateral shoots bore opposite pairs of leaves for at least the first two nodes. The main changes in a 3-month-old seedling were: (a) the main stem began to bend over, and the new leaves produced beyond the third or fourth node were subopposite and later alternate, with the same change in lateral shoots, and (b) secondary lateral shoots appeared at the cotyledon nodes and the first two leaf nodes. These grew out horizontally between the primary lateral shoot above and the cotyledon or leaf below, and further emphasized the procumbent growth form which had begun to develop.

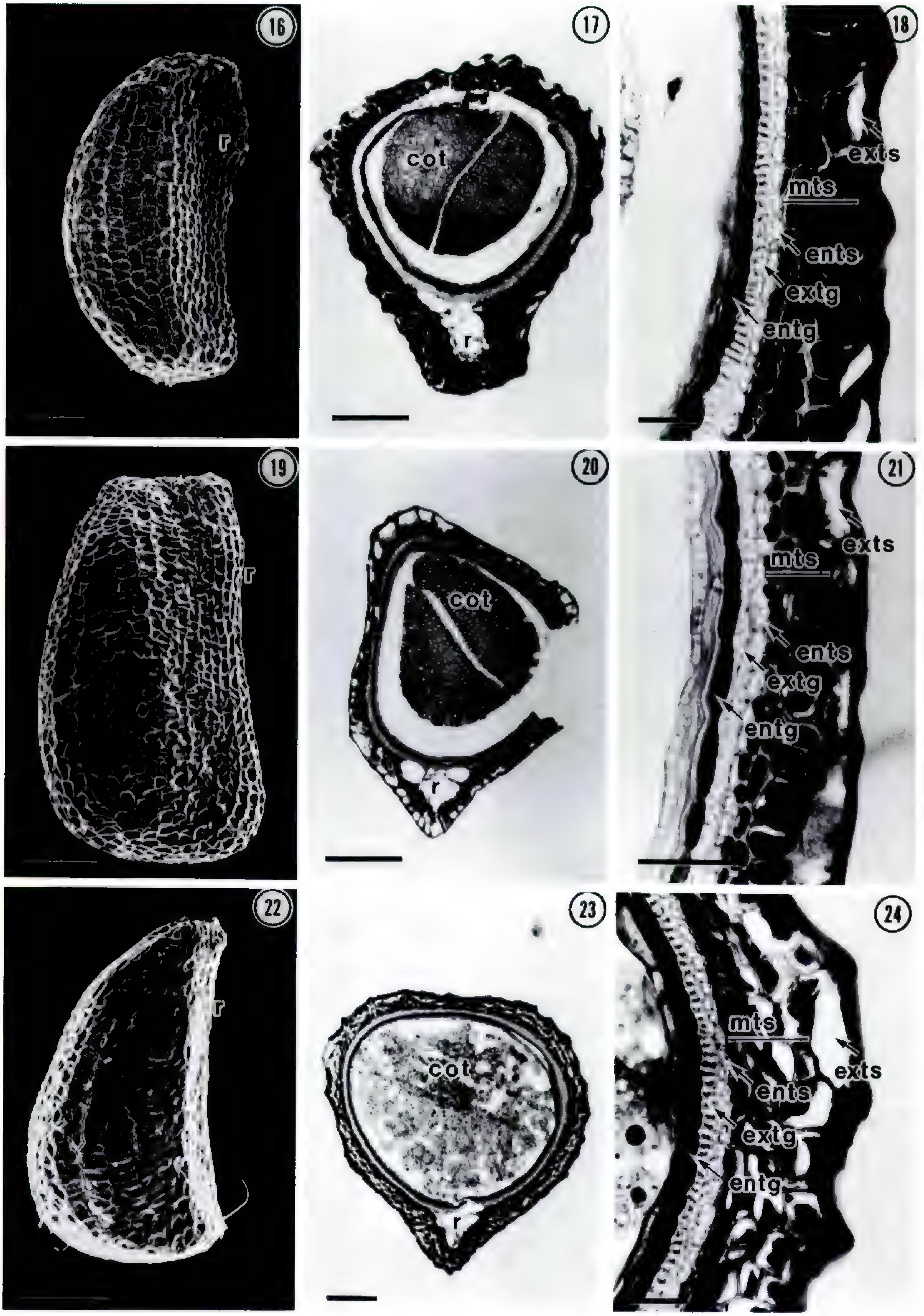
The main conclusion from these observations is that seedlings of the South Pacific fuchsias begin with opposite and decussate leaves, but only in *Fuchsia cyrtandroides* is this leaf arrangement found in the adult. If opposite leaves are considered primitive in the Onagraceae (fide Raven in Cronquist, 1981), then *F. cyrtandroides* may be plesiomorphic in this respect. Second, the presence of secondary shoots in the axils of *F. procumbens* seedlings is an example of the basis for Willis's (1925) statement that many fuchsias "show two buds in each axil, one above the other."

Leaf Anatomy and Foliar Flavonoids

The leaf anatomy of *F. excorticata* (Table 3) shows no xerophytic features. A cuticle is either absent or poorly developed, and the stomata are not sunken or otherwise protected. The outstanding feature is the absence of chlorophyll in the spongy parenchyma, which makes up the lower ⅔ of the leaf, leading to the silvery color of the lower leaf surface (Suckling, 1914).

In *Fuchsia perscandens,* the main differences in leaf anatomy from *F. excorticata* are that the palisade tissue is only 1-layered in the much smaller and thinner leaf and that chlorophyll is present in the spongy parenchyma (Table 3).

Foliar flavonoids have been well characterized for all sections and most species of *Fuchsia,* as a result of the broad surveys by Averett & Raven (1984) and Averett et al. (1986). Williams et al. (1983) also analyzed a set of six *Fuchsia* cultivars, and Williams & Garnock-Jones (1986) examined the different members of section *Skinnera.* These results show that all species of *Fuchsia* contain flavonol-3-O-glucosides based on quercetin and kaempferol. Flavone glycosides occur in only nine species belonging to five sections, but all four South Pacific species of *Fuchsia* are characterized by the presence of flavones. Furthermore, flavone sulphates, which are found nowhere else in the genus, are known from all South Pacific species of *Fuchsia* except *F. cyrtandroides.*



FIGURES 16–24. Morphology and anatomy of mature seeds of the *Fuchsia* sect. *Skinnera*.—16, 19, 22. Scanning electron micrographs of entire seeds (scale = 200μ).—17, 20, 23. Transverse sections of entire seeds (scale = 100μ).—18, 21, 24. Transverse sections of seed coat (scale = 20μ). 16–18. *F. cyrtandroides*. 19–21. *F. excorticata*. 22–24. *F.*

CYTOLOGY

Chromosome counts have been made for all South Pacific taxa of *Fuchsia* (Table 4; Figs. 29–32), showing them to be uniform in chromosome number, with $n = 11$. This is the basic chromosome number for both the genus (Berry, 1982) and the family (Raven, 1979a). Meiosis was also analyzed in several artificial hybrids involving *F. procumbens* and the other two New Zealand species (Table 4; Figs. 30–32). Pairing was normal except in one individual of *F. procumbens* × *F. excorticata*, in which there were two univalents.

ARTIFICIAL CROSSES

A series of artificial crosses was made in Lincoln, outside Christchurch, New Zealand, with results presented in Table 5. The crosses in 1951–1952 and 1990–1991 were done by E. Godley, and those in 1969–1973 were done by his assistant, Diane H. Smith.

Fuchsia procumbens × *F. excorticata* (Table 5). In four separate crosses, 29 flowers gave 18 fruits and yielded 783 seeds. Cross 1969/1 differed from the others in that the pollen (collected on 14 October) was used over five days on three occasions. Pollen used on 15 October to pollinate eight flowers produced two fruits; when used on 16 October, one fruit resulted from three flowers pollinated; and on 20 October, all four flowers pollinated produced fruit, which may indicate a higher viability for the older pollen. The cross with *F. excorticata* as the female parent (cross 1952/1) gave a much greater number of seeds per fruit than when *F. procumbens* was the female, presumably because of the much greater number of ovules in *F. excorticata*. Likewise, fruit size in crosses where *F. procumbens* was the female parent was much greater than when *F. excorticata* was the pollen recipient, reflecting the much larger size of *procumbens* fruits. When female plants of *F. procumbens* were used as pollen recipients, seed germination was much higher than when hermaphroditic flowers were used from hermaphrodite-male plants.

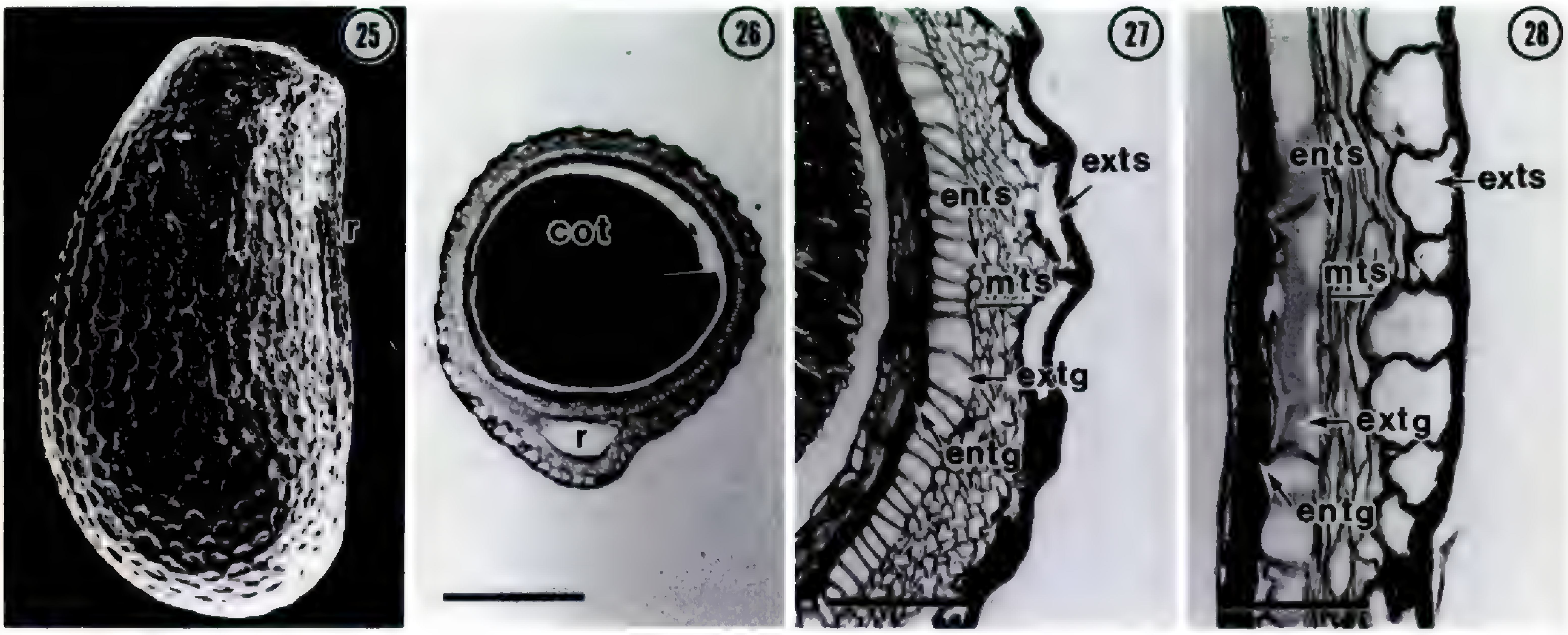
Seedlings from cross 1951/6 (*procumbens* × *excorticata*) were given to H. H. Allan in Wellington, who wrote, "In their second year the plants are erect shrubs with spreading branches" (Allan, 1961). The leaves of an eight-year-old plant grown at Lincoln were intermediate in size and shape between the parents. The flowers showed a slight swelling at the base of the floral tube, derived from *F. excorticata*, and the calyx lobes were reflexed as in *F. procumbens*. As females of *F. procumbens* are heterozygous for the dominant gene for male sterility, and hermaphrodites of *F. excorticata* are homozygous recessive, these crosses gave plants with either female or hermaphrodite flowers. In both types, the style protruded for varying lengths beyond the staminodes or stamens. N. T. Moar reported in October 1958 that the total pollen grains on each of two slides from a hermaphrodite were 63 and 121, with viable grains at 16% and 17%, respectively. A sample from *F. procumbens* (Ngawae) gave 66% viable pollen.

When plants of cross 1969/1 were four years old, leaf blade dimensions ranged from 3.0 × 1.5 cm to 4.7 × 3.0 cm (a leaf from each individual was pressed and mounted as CHR 194409 A–H). The first flowers were observed on 18 January 1974, and during the first flowering season, there were 20 female and 8 hermaphrodite plants. The number of petals on these plants varied from zero to four. An hermaphroditic plant showed either perfect pairing at meiosis or else ten bivalents and two univalents (Figs. 31, 32; Table 4).

Fuchsia procumbens × *F. perscandens* (Table 5). In cross 1969/2, pollination was spread over eight days, but fresh pollen from a nearby source was used for each batch. Approximately 41% of the seed from the 15 fruits that were produced were separated as "bad," and these 207 seeds when sown separately gave only 12 seedlings. The remaining 294 seeds gave 71.7% germination. In four-month-old plants the main central stem had turned over and was prostrate, at least near the tip. As with *F. procumbens*, the secondary branches (up to 5–7) became prostrate and much longer (up to 60 cm long). In a few five-month-old plants, the secondary prostrate branches were only about 30 cm long, and there were several secondary shoots up to 8 cm high on the crown of the plant. The first hybrid to flower was observed almost precisely one year after seeds were sown, and the second plant flowered one month later. Both plants bore female flowers, similar to *F. procumbens* × *F. excorticata*. Meiosis in a later-flowering hermaphroditic individual showed 11 bivalents (Fig. 30; Table 4).

←

perscandens. Abbreviations: cot, cotyledons; entg, endotegmen; ents, endotesta; extg, exotegmen; exts, exotesta; mts, mesotesta; r, raphe. Microphotographs by H. Tobe.



FIGURES 25–28. Morphology and anatomy of mature seeds of *Fuchsia procumbens.*—25. Scanning electron micrograph of entire seed (scale = 200μ).—26. Transverse section of entire seed (scale = 500μ).—27. Transverse section of seed coat (scale = 100μ).—28. Longitudinal section of seed coat (scale = 100μ). Abbreviations same as in Figures 16–24. Microphotographs by H. Tobe.

Fuchsia excorticata × *F. perscandens* (Table 5). In cross 1969/3, although 16 flowers were pollinated with fresh pollen in three batches, only one fruit was collected, and it was suspected that birds had eaten any others. Of the 76 seeds sown on 26 February 1970, 42 had germinated by 15 March. However, most young seedlings died by 1 April, and the only survivors were three seedlings which came from seeds germinated after this time. These plants reached heights of 50, 42, and 27 cm by September 1970.

In cross 1990/1, the contents of a whole fruit were sown, but only two seeds germinated, and these were left growing at Lincoln (accession #G283/91); leaves of the hybrid and parents are shown in Figure 33. Cross 1991/1 was the most successful of this series, with 350 seeds obtained from five of the ten fruits, and 21 plants of the numerous progeny left growing at Lincoln (accession #G54/92).

Fuchsia cyrtandroides × *F. procumbens* and *F. perscandens.* Although very few flowers of *F. cyrtandroides* were available to pollinate, crosses with both *F. procumbens* and *F. perscandens* did produce numerous seeds, and in both cases the large ovule numbers of the female parent are reflected in large seed numbers. Robust hybrid plants were obtained from the cross of *F. perscandens* × *F. cyrtandoides,* and these later flowered.

NATURAL HYBRIDS

Fuchsia excorticata and *F. perscandens* have the same geographical range except that *F. perscandens* is not found in the far north nor on Chatham, Stewart, or Auckland Islands. Throughout the range of *F. perscandens,* the two species overlap ecologically so that natural hybridization is possible, and plants intermediate between the two species are regularly collected. R. L. Bieleski reported his impression of the situation of these two species and numerous intermediates after a trek along the Heaphy Track, between Karamea and Bainham on the South Is-

Table 3. Features of the leaf anatomy in South Pacific species of *Fuchsia.*

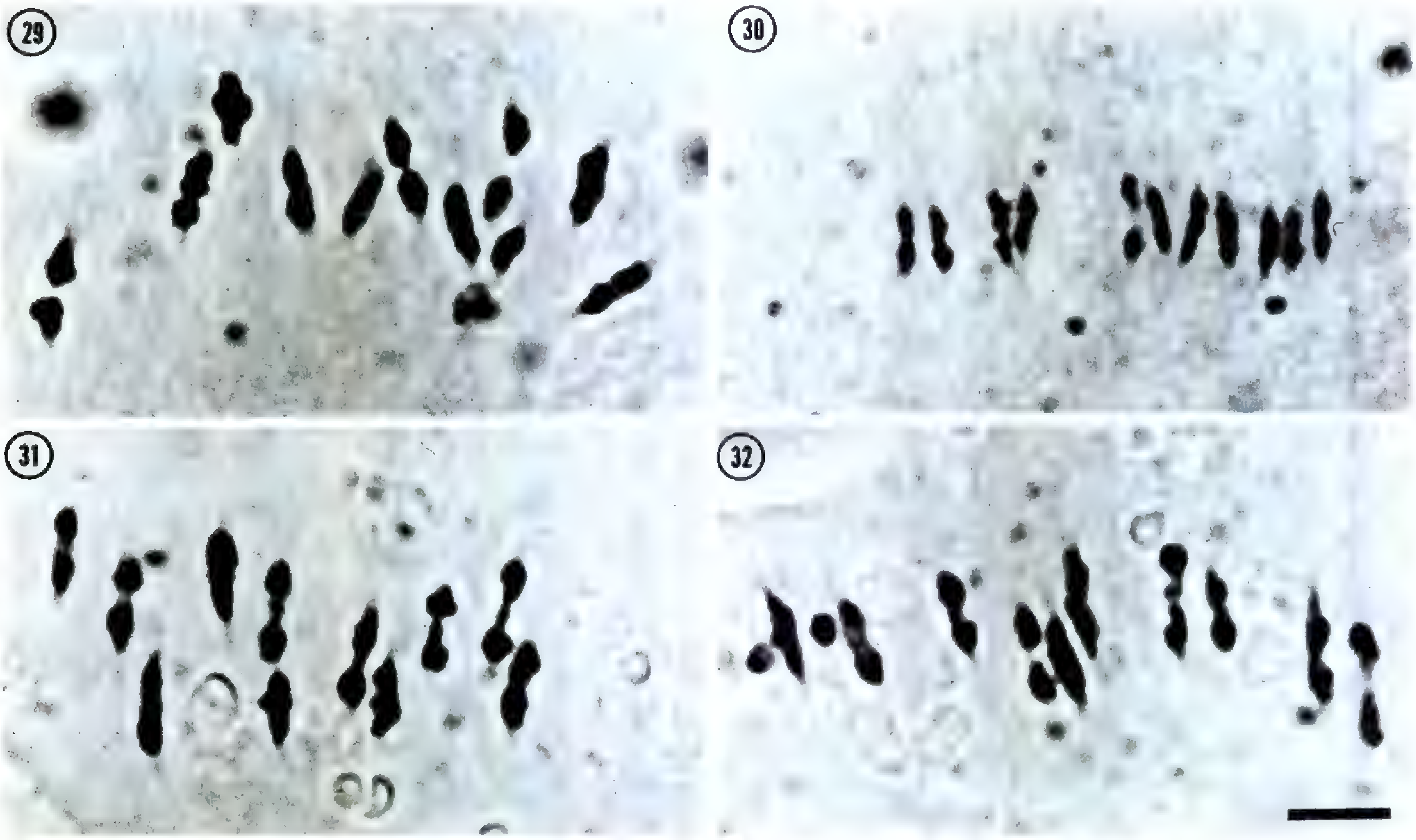
	Upper epidermis	Palisade tissue	Spongy tissue	Lower epidermis
F. excorticata	2-layered, stomata absent	2 or 3 layers	Chloroplasts absent	1 layer with stomata
F. cyrtandroides	3- or 4-layered, stomata absent	Up to 4 layers	Chloroplasts absent	1 thin layer with stomata
F. perscandens	1 layer, stomata absent	1 layer	Chloroplasts present	1 thin layer with stomata
F. procumbens	1 large layer, stomata absent	1 layer	Chloroplasts present	1 layer?, stomata undulate

Table 4. Chromosome numbers in the South Pacific species of *Fuchsia.*

Taxon	Chromosome number	Voucher, source locality
Fuchsia excorticata	$n = 11$	*CHR 200293* (Botanic Gardens, Christchurch)[1]
Fuchsia perscandens	$n = 11$	*CHR 200301* (Riccarton Bush, Christchurch)[1]
Fuchsia ×*colensoi*	$n = 11$	*CHR 200300* (Riccarton Bush, Christchurch)[1]
Fuchsia cyrtandroides	$n = 11$	*Carlquist 653* at DS (Aorai, Tahiti)[2]
	$n = 11$	*Plant G9804/6*[3]
Fuchsia procumbens	$n = 11$, $2n = 22$	*CHR 200302-3* (Cultivated plants, Auckland)[1]
Fuchsia procumbens × *F. excorticata*	$n = 11$, $n = 10$ bivalents and 2 univalents	*Cross 1969/1* from Table 5[3]; *CHR 194409 A–H*
Fuchsia procumbens × *F. perscandens*	$n = 11$	*Plant G8968* from Cross 1969/2 in Table 5[3]

[1] Report by Beuzenberg & Hair (1959).
[2] Counted by P. H. Raven.
[3] Counted by E. J. Beuzenberg.

land, in February 1972: small-leaved, low scrambling plants of *F. perscandens* were found along the alluvial river flats; large silvery-leaved shrubs of *F. excorticata* occurred close to *F. perscandens* but were more numerous on limestone knobs on higher ground; and a variety of "*colensoi*" plants with intermediate growth forms and leaves occurred in coastal flats and in the coastal hedge facing the sea, becoming more and more *excorticata*-like as the path changed to traverse granite heads and valleys



FIGURES 29–32. Meiotic chromosomes in *Fuchsia cyrtandroides* and in hybrids between *F. procumbens* and *F. excorticata* and *F. perscandens.* Scale bar = 5 μ; photographs by E. J. Beuzenberg.—29. Meiosis in *F. cyrtandroides,* with 11 bivalents, from accession #G9804/6 at D.S.I.R., Lincoln, New Zealand.—30. Meiosis in an hermaphrodite plant of *Fuchsia procumbens* × *F. perscandens,* showing 11 bivalents, from cross 1969/2, plant G8968.—31. Meiosis in a plant of *Fuchsia procumbens* × *F. excorticata,* showing 11 bivalents, from cross 1969/1 (Table 5), accession # G9140/30 at D.S.I.R., Lincoln, New Zealand.—32. Meiosis in a plant of *Fuchsia procumbens* × *F. excorticata,* showing 10 bivalents and 2 univalents, from cross 1969/1 (Table 5), accession # G9140/30 at D.S.I.R., Lincoln, New Zealand.

Table 5. Artificial crosses between South Pacific species of *Fuchsia*.

Cross	Date	Parents (female first)	Number of flowers pollinated	Number of fruit set	Fruit length (mm)	Seeds per fruit	Seeds germinated/sown	Voucher specimens
1951/6	10 Nov.	*F. procumbens* (female) × *F. excorticata* (hermaphrodite)	7	6	9–10	20–41	69/177	CHR 79041 CHR 191429–30 CHR 191432–33
1951/11	19 Dec.	*F. procumbens* (hermaphrodite-male) × *F. excorticata* (hermaphrodite)	5	4	20–27	27–55	2/155	
1952/1	29 Jan.	*F. excorticata* (female) × *F. procumbens* (hermaphrodite-male)	2	1	?	104	3/104	
1969/1	15, 16, 20 Oct.	*F. procumbens* (female) × *F. excorticata* (hermaphrodite)	15	7	10–20	23–65	208/357	CHR 191308–12 CHR 365025 CHR 465324
1969/2	3, 4, 11 Nov.	*F. procumbens* (female) × *F. perscandens* (hermaphrodite)	16	15	10–16	13–49	223/294 + 207[1]	
1969/3	4, 5, 11 Nov.	*F. excorticata* (female) × *F. perscandens* (hermaphrodite)	16	1	10	76	42/76	
1970/1	24 Mar.	*F.* ×*colensoi* (hermaphrodite) self-pollinated					76/242	CHR 325596 A–D
1973/1	26 Feb.	*F. cyrtandroides* × *F. procumbens* (male)	4	2		273–289	0/562	
1973/2	11 Sep.	*F. perscandens* (hermaphrodite) × *F. cyrtandroides*	1	1		152	N.A.[2]/152	CHR 465323 A–C
1990/1	7 Nov.	*F. excorticata* (female) × *F. perscandens* (hermaphrodite)	3	2			2/(1 fruit)	CHR 484724
1991/1	8 Nov.	*F. excorticata* (female) × *F. perscandens* (hermaphrodite)	10	10	11–13		numerous/350 (5 fruits)	CHR 484725

[1] The second group of seeds was separated before being sown as being malformed and unlikely to germinate; see text for additional discussion.
[2] Number not recorded, but some seed did germinate and grew to flowering stage.

FIGURE 33. Leaves of cross 1990/1 (Table 5) between *Fuchsia excorticata* and *F. perscandens*, showing the range of leaf sizes for the two parents and the F1 hybrids. Top row, *F. excorticata*; middle row, *F. excorticata* × *F. perscandens*; bottom row, *F. perscandens*.

with coarse sand (letter to E. Godley, 16 Feb. 1972).

Allan (1961) wrote that "the species [*F. colensoi*] is maintained meantime, as many of the localities are far outside the known occurrences of *F. perscandens*." His main reason for this statement is that he accepted Cheeseman's (1906, 1925) record of *F. colensoi* from the Northern Wairoa, North Auckland. However, Cheeseman's specimen was actually *F. perscandens*, which was not recognized in his time.

Plants intermediate between *F. excorticata* and *F. perscandens* were first classified as *F. colensoi* Hook. f. Later, Allan (1927) described them as *F.* ×*experscandens* and wrote, "Where we have found this species [*F. perscandens*] we have observed it to hybridize with *F. excorticata* if that is also present. A polymorphic swarm is produced, many of the forms of which—their origin being unknown—might be referred to *F. colensoi*. Whether one or more shrubby jordanons exist, or whether *F. colensoi* is purely a series of hybrid forms remains to be investigated." But Allan's alternatives are not mutually exclusive. Both crossing and selfing could be at work. That plants referable to *F. colensoi* can arise as hybrids is shown by our artificial crosses. The hybrids are all hermaphrodite if both parents are hermaphrodite, or if the cross is between an hermaphrodite and a female the progeny will be a mixture of these types. There are opportunities therefore in the F_1 generation for outcrossing and for selfing, the former contributing to polymorphic forms, the latter to more inbred lines.

Field and experimental evidence shows that *F. colensoi* should not be maintained as a species, but that it arises as a fertile natural hybrid between *F. perscandens* and *F. excorticata*. Backcrosses with both parents appear to occur frequently, although *F.* ×*colensoi* is much more common and widespread than *F. perscandens*. From historical records, it appears that *F. perscandens* was much more common until colonization and deforestation over the last centuries led to the demise of the mainly lowland forested habitats preferred by this species. As a mostly self-supporting bush to a small tree, *F.* ×*colensoi* is apparently much better suited to disturbed habitats than is *F. perscandens*, and this may help explain why it is currently more common and widespread. Wilson (1982, 1987) reported *F.* ×*colensoi* from Stewart Island, where *F. excorticata* is known to grow but *F. perscandens* is not; he suggested that birds could have transported seeds of hybrid plants from the mainland.

Cross 1970/1 involved self-pollinated flowers of *Fuchsia* ×*colensoi* (hermaphrodite) collected on the Otago Peninsula by P. Raven (*Raven 25113*, MO). From 242 seeds 76 plants were reared. Leaf blade dimensions showed little variation and ranged in size from 26 × 20 mm to 56 × 36 mm (leaf samples were taken and mounted as *CHR 325596 A–D*). The smallest leaf approached the presumed *F. perscandens* parent, while most of the plants come within the range of *F.* ×*colensoi*, and none approached the normal dimensions of *F. excorticata* (see Fig. 33).

A chloroplast DNA restriction site analysis that included samples of *F. excorticata*, *F. perscandens*, and *F.* ×*colensoi* (Sytsma et al., 1991) remarkably found no differences in the chloroplast genome among the three, which suggests a high level of introgression. It is possible that the maternal genome of the more widespread *F. excorticata* has swamped out that of the much rarer *F. perscandens*.

Evolution and Biogeography

The South Pacific species of *Fuchsia* are key elements to understanding evolution in the genus because of their unusual combination of characters and their widely disjunct distribution. Several distinct lines of evidence support the early separation of the South Pacific species from the American fuchsias. Sections *Procumbentes* (see below) and *Skinnera* are the only sections in which all species have flavones, and all of the South Pacific species except for *F. cyrtandroides* have flavone sulphates that occur nowhere else in the genus. Furthermore, these are the only two sections known to have male sterility controlled by a dominant gene, and all four species share the apomorphy of blue pollen. Other distinguishing characters of the South Pacific fuchsias are the greenish flowers and marked floral color changes during anthesis (all three species of section *Skinnera*), the strongly reduced or missing petals, and the wide variety of habit types from trees to small creepers. Chloroplast DNA restriction site analysis of 18 species from ten sections of *Fuchsia* indicated that among those lineages that have persisted until the present, sections *Procumbentes* and *Skinnera* were the first ones to diverge within the genus and that together they form the sister group to all other *Fuchsia* sections (Sytsma & Smith, 1988, 1992).

Fossil pollen records of *Fuchsia* from Oligocene deposits in Australia (Berry et al., 1990), New Zealand (Pocknall & Mildenhall, 1984), and Argentina (E. Romero, pers. comm.), as well as the South American center of distribution for the genus, suggest that *Fuchsia* evolved in subtropical forests of the southern hemisphere during the early to middle Tertiary (Berry et al., 1990). Until the late Eocene or perhaps into the early Oligocene, present-day portions of Australia, Antarctica, and southern South America formed contiguous land masses, with more or less direct overland migrations possible between them (Kennett, 1980; Zinmeister, 1987). Based on the known fossil records of *Fuchsia* in Australia as early as 35 mya, it may be more realistic to estimate the initial diversification of the South Pacific and the New World fuchsias at closer to 50 mya (Sytsma et al., 1991).

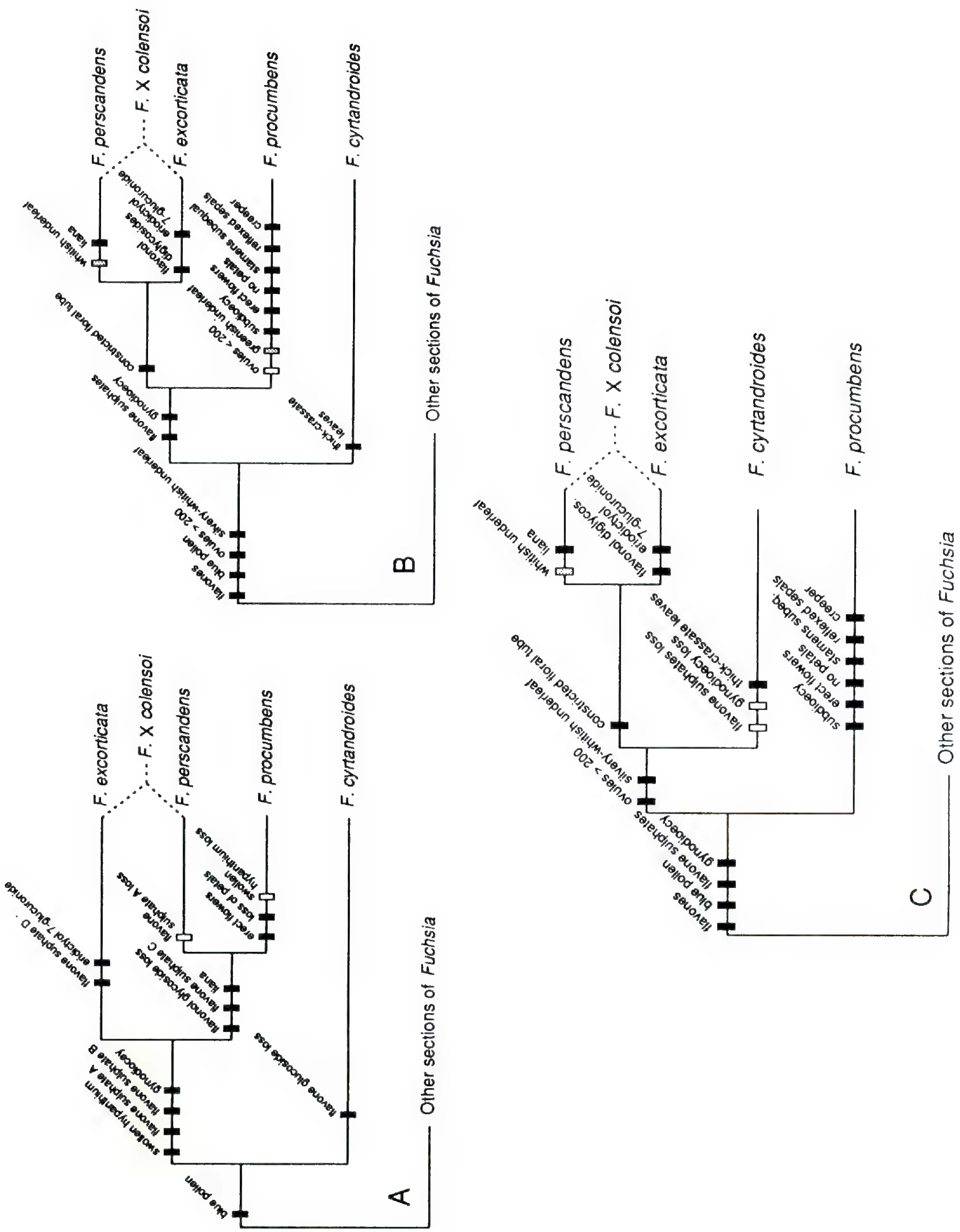
Ancestors of the South Pacific fuchsias were likely present in Antarctica and Australia during the Eocene, when both land masses were in close proximity and moist subtropical to temperate forests were the dominant vegetation type. By the Oligocene, *Fuchsia* was already widely scattered in eastern Australia (Berry et al., 1990). Sometime after the mid-Miocene, however, when the last known fossil records are known from Australia, it may have gone extinct there, a victim of increasing warmth and dryness as the continent rafted northwards (Barlow, 1981). New Zealand, on the other hand, has apparently maintained mesic conditions for much of its history since it separated from Australia and Antarctica about 80 mya (Raven, 1979b). Since the oldest fossil grains of *Fuchsia* from New Zealand date from the Late Oligocene, roughly 30 mya (Mildenhall, 1980), it is likely that the genus had to disperse there from Australia across the Tasman Sea, following the prevailing westerlies.

Examination of different kinds of characters in the four South Pacific species of *Fuchsia* does not make their relationships immediately clear. In its tree habit and strongly bicolored leaves, *F. cyrtandroides* from Tahiti is most similar to the New Zealand tree fuchsia, *F. excorticata*. On the other hand, it is the only species in the group with opposite leaves as an adult and that lacks both flavone sulphates and male sterility. Since Tahiti is a young volcanic island approximately 2 MY old (Dymond, 1975), *Fuchsia* either arrived there recently via long-distance dispersal from a source such as New Zealand, as postulated by Carlquist (1967) and others (Raven, 1979a; Fleming, 1976; Godley, 1979; Berry, 1982), or was present on older islands in the Pacific that have since subsided. *Fuchsia procumbens* from New Zealand is the most distinctive species of the group, although it most closely resembles *F. perscandens* vegetatively. *Fuchsia perscandens* has flowers very similar to *F. excorticata*, but differs markedly in its bushy or lianoid habit.

Williams & Garnock-Jones (1986) attempted the first phylogenetic analysis of the South Pacific species, using mostly leaf flavonoids with some habit and flower characters. Their results produced a single, shortest cladogram (Fig. 34A) in which *F. cyrtandroides* was the sister group of the New Zealand species, and *F. excorticata* the sister species of the pair *F. procumbens* and *F. perscandens*. Crisci & Berry (1990) reanalyzed the same taxa with a

→

Figure 34. Published trees of the South Pacific species of *Fuchsia* derived from cladistic analysis of morphology and flavonoids.—A. The single most parsimonious tree obtained by Williams & Garnock-Jones (1986).—B, C. The two most parsimonious trees obtained by Crisci & Berry (1990). Note that the tree in B has the same topology as that of A. Reprinted with permission of *Systematic Botany*.


A
F. excorticata
F. X colensoi
F. perscandens
F. procumbens
F. cyrtandroides
Other sections of Fuchsia
blue pollen
swollen hypanthium
flavone sulphate A
flavone sulphate B
gynodioecy
flavone glucoside loss
flavonol glycoside loss
flavone sulphate C
liana
flavone sulphate A loss
erect flowers
loss of petals
swollen hypanthium loss
flavone sulphate D
eriodictyol 7-glucuronide
B
F. perscandens
F. X colensoi
F. excorticata
F. procumbens
F. cyrtandroides
Other sections of Fuchsia
flavones
blue pollen
ovules > 200
silvery-whitish underleaf
flavone sulphates
gynodioecy
constricted floral tube
whitish underleaf
liana
flavonol diglycosides
eriodictyol 7-glucuronide
ovules < 200
greenish underleaf
subdioecy
erect flowers
no petals
stamens subequal
reflexed sepals
creeper
thick-crassate leaves
C
F. perscandens
F. X colensoi
F. excorticata
F. cyrtandroides
F. procumbens
Other sections of Fuchsia
flavones
blue pollen
flavone sulphates
gynodioecy
ovules > 200
silvery-whitish underleaf
constricted floral tube
whitish underleaf
liana
flavonol diglycos.
eriodictyol 7-glucuronide
flavone sulphates loss
gynodioecy loss
thick-crassate leaves
subdioecy
erect flowers
no petals
stamens subeq.
reflexed sepals
creeper

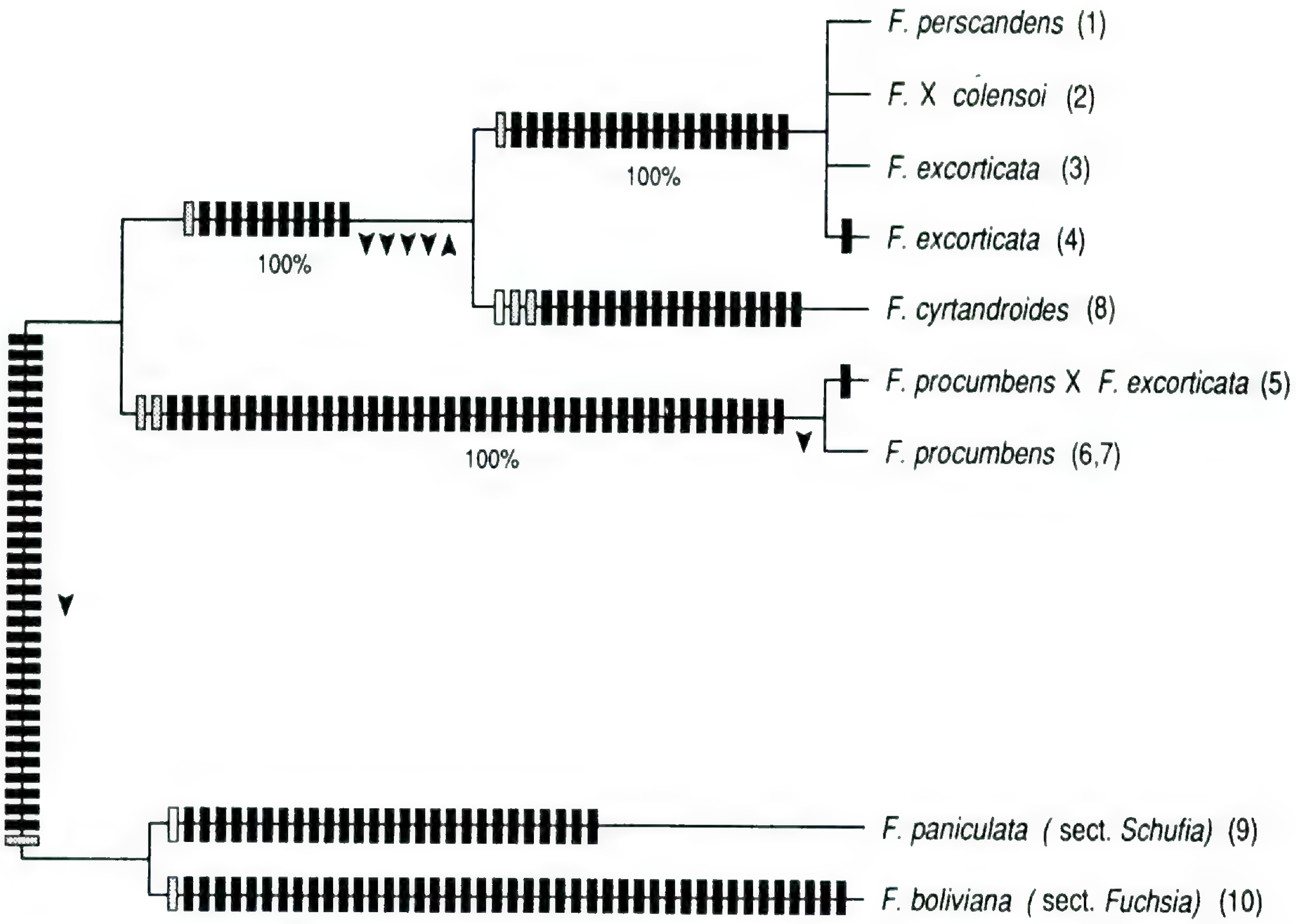


FIGURE 35. The most parsimonious cladogram of the South Pacific species of *Fuchsia* obtained by Sytsma et al. (1991) from chloroplast DNA restriction site mutations. Solid bars represent uniquely occurring site changes. Gray bars represent likely homoplastic site changes (convergent losses, or gains followed by losses), and open bars represent unlikely changes (convergent gains, or losses followed by gains). Downward arrows represent deletions and the upward arrow an insertion (these were not used to generate the tree). For the resolved branches confidence levels are 100% by Wagner bootstrap analysis. Reprinted with permission of *Systematic Botany*.

broader interpretation of flavonoid characters and additional characters from leaves and sexual systems. They arrived at two equally parsimonious trees, both differing from the previous study by placing *F. perscandens* closest to *F. excorticata* rather than to *F. procumbens*. The first cladogram (Fig. 34B) resembled Williams and Garnock-Jones's results in placing *F. cyrtandroides* as the sister species of the other three, while in the second (Fig. 34C), *F. procumbens* was the sister group of the other taxa, with *F. cyrtandroides* the sister species of *F. excorticata* and *F. perscandens*. These differing cladograms were largely the result of the difficulty in polarizing characters such as the presence/absence of flavone sulphates and male sterility.

Sytsma et al. (1991) employed the technique of chloroplast DNA restriction site analysis to examine the four South Pacific species of *Fuchsia*, two interspecific hybrids in this group, and two outgroup species from other sections of the genus. Their phylogenetic analysis of the results of over 790 restriction sites examined within each accession produced a single, highly reliable, and statistically significant cladogram (Fig. 35) that agreed with the second tree of Crisci & Berry (1990, Fig. 34C above), in which *F. procumbens* is the sister group to the other South Pacific species, and *F. cyrtandroides* is the sister group of *F. excorticata* and *F. perscandens*. The chloroplast genomes of *F. excorticata* and *F. perscandens* were virtually indistinguishable, arguing for either a very close relationship between the two or else the swamping of the maternal genome of one species by hybridization and unidirectional backcrossing to the other.

This robust phylogenetic tree of the South Pacific *Fuchsia* species from detailed molecular data supports an early divergence of *Fuchsia procumbens* from the other South Pacific species. This lends strong support to the recognition of a new section *Procumbentes* to accommodate *F. procumbens* as the sister group to the three species of section *Skinnera*. A relatively ancient separation of these two South Pacific lineages is consistent with the early presence of *Fuchsia* fossils in New Zealand and Australia. Within section *Skinnera*, the large number of differences in cpDNA between *F. cyrtan-*

droides and the *F. excorticata–F. perscandens* species pair suggests that these two clades diverged about 10 mya, long before Tahiti emerged from the Pacific ca. 2 mya (Sytsma et al., 1991). One explanation is that *F. cyrtandroides* or its immediate ancestor evolved for a considerable amount of time in New Zealand or on another island, then became extinct there after dispersing to Tahiti or older, now submerged islands in the mid-Pacific.

The study by Sytsma et al. (1991) also has important implications for character evolution in the South Pacific sections. When the morphological, flavonoid, and sexual characters are superimposed on the molecular-derived cladogram, gynodioecy appears as the ancestral condition to all South Pacific species, with *F. procumbens* becoming subdioecious through the subsequent development of female sterility, and *F. cyrtandroides* becoming hermaphroditic through the secondary loss of male sterility. Likewise, the absence of flavone sulphates in *F. cyrtandroides* must be viewed as a secondary loss, rather than a primitive absence shared with the New World species. Lastly, the shrub or tree habit in *F. cyrtandroides* and *F. excorticata* should not be interpreted as a shared, derived character, but rather as the generalized condition in the genus. The unusual, procumbent habit of *F. procumbens* and the lianoid habit of *F. perscandens*, on the other hand, are best viewed as independently derived conditions from shrubby ancestors.

The progression of studies outlined above dramatically illustrates the perils of making conclusions about biogeography and character evolution without an exhaustive analysis of several kinds of evidence, including molecular studies. There is already evidence for extensive homoplasy in *Fuchsia* in characters such as leaf position, nectary types, breeding systems, floral color changes, loss of petals, polyploidy, and pollen morphology and ultrastructure. For these reasons, considerable caution will be needed in interpreting relationships and phylogenetic trends in the other parts of the genus.

Taxonomic Section

Because of the distinctiveness of *Fuchsia procumbens* and its basal position within the South Pacific clade, we choose to recognize it as a new section. It is distinguished morphologically by a very different fruit type from section *Skinnera*, the absence of petals, erect flowers that do not undergo marked changes in flower color during anthesis, and a unique habit type in the genus. These are characters that would readily be recognized at the sectional level for any of the American species. The ease of hybridization between *F. procumbens* and members of section *Skinnera* is not a strong argument to maintain them as a single section, as it is easy to produce intersectional hybrids between many of the American sections, and members of both section *Skinnera* and *Procumbentes* have successfully produced hybrid progeny when crossed with American species in the greenhouse.

Key to the South Pacific Taxa of *Fuchsia*

1. Flowers erect, without petals; procumbent subshrubs (section *Procumbentes*) .... 1. *F. procumbens*
1. Flowers pendulous, with small, lanceolate petals; trees, shrubs or lianas (section *Skinnera*) 2
2. Plant a liana or densely branched, scandent to compact shrub; leaves suborbicular to broadly ovate, pale green below, secondary veins 3–5 per side, petiole ½ to 1 times the blade length .... 4. *F. perscandens*
2. Plant a tree or erect to sometimes scandent shrub; leaves elliptic-ovate to lanceolate, whitish to silvery below, secondary veins 6–12 per side, petiole mostly less than ½ the blade length 3
3. Floral tube cylindrical, the base not bulbous; leaves mostly opposite, the apex rounded or obtuse; Tahiti .... 2. *F. cyrtandroides*
3. Floral tube strongly constricted in the middle, the base bulbous; leaves mostly alternate, the apex acute to acuminate; New Zealand 4
4. Plant a tree with distinct trunk; leaves mostly > 5 cm long, secondary veins 9–12 per side .... 3. *F. excorticata*
4. Plant a shrub, much branched from the base, branches often scandent; leaves mostly < 5 cm long, secondary veins 6–8(9) per side .... 5. *F.* ×*colensoi*

Fuchsia section **Procumbentes** E. J. Godley & P. E. Berry, sect. nov. TYPE: *Fuchsia procumbens* R. Cunningham ex A. Cunningham.

Sectioni *Skinnerae* affinis, a qua differt habitu subligneo serpentique, floribus erectis subdioeciis, sepalis reflexis, petalis nullis, staminibus fere uniseriatis, ovario 50–230-ovulato, fructus loculis cavis.

Trailing subshrubs. Leaves alternate, suborbicular to broadly ovate, firmly membranous, weakly bicolored, sinuate- or serrulate-margined, the blade shorter than the petiole. Subdioecious (plants male, female, or sometimes male-hermaphrodite), the flowers axillary and erect, females smaller than males or male-hermaphrodites. Floral tube cylindrical, light green in bud, pale orange-yellow at anthesis; nectary a smooth band lining the inner, basal part of the tube. Sepals completely reflexed soon after anthesis. Petals absent. Stamens uniseriate or weakly biseriate; pollen bright blue, 2-aperturate, with beaded to nearly smooth viscin threads. Stigma capitate and somewhat 4-angled when functional. Ovary green, with 50–230 biseriate ovules.

Berry ovoid-oblong to subglobose, 10–25 × 8–16 mm, bright red with a pruinous covering when ripe, firm-walled, with hollow locules; seeds oblong to (ob-)ovoid, 1.7–2.8 mm long, 1.3–1.6 mm wide. Gametic chromosome number $n = 11$.

Distribution (Fig. 6). Far northern districts of the North Island of New Zealand.

1. **Fuchsia procumbens** R. Cunningham ex A. Cunningham, Ann. Nat. Hist. 3: 31. 1839. TYPE: New Zealand. North Island: around the village of Matauri, on the E coast opposite the Cavallos Isles, inhabiting the sands immediately above the range of the tide, Mar. 1834, (female), *Richard Cunningham* (holotype, K).

Fuchsia kirkii Hooker f., Icon. Pl. 11: 66, pl. 1083. 1871. TYPE: New Zealand. Great Barrier Island: near the sea, Dec. 1867, (male), *Thomas Kirk 62* (holotype, K).

Densely branched, procumbent subshrubs, with slender, trailing main stems 1–2(–8) mm thick, to 2 m long, often rooting along the ground and with exfoliating bark when old; young branchlets very slender and glabrous, ascending near the tips, sometimes scandent on tree trunks or in dense brush. Leaves alternate, firm-membranous, suborbicular to broadly ovate, 5–28 mm long, 3–27 mm wide, apex obtuse to rounded, base subcordate to truncate, pale green to purple-flushed on both surfaces, slightly paler beneath; margin sinuate to serrulate with prominent glandular teeth, strigulose along the nerves, secondary veins (3)4 or 5(6) per side, lower order venation inconspicuous. Petioles slender, strigulose, longer than the blade, 5–50 mm long. Stipules minute, narrowly lanceolate, 0.3–0.9 mm long, ca. 0.2 mm wide at the base, caducous.

Subdioecious; flowers axillary and erect on pedicels 3–10 mm high. *Staminate or perfect flowers*: ovary cylindrical, 3–6 mm long, 2–2.8 mm thick, green and pruinous, 4-locular, each locule with 15–55 ± biseriate ovules. Floral tube cylindrical, 7–11 mm long, 5.2–7.5 mm wide at the rim, light green in bud, pale orange-yellow at anthesis, in some cases turning slightly darker with age; nectary 2–4 mm high, lustrous yellow-orange. Sepals lanceolate, acute, 7–9 mm long, 2.7–3.8 mm wide at the base, completely reflexed soon after anthesis, light to dark purple on the exposed, inner surface except at the green base, green on the hidden, outer side. Petals absent. Filaments equal or subequal, 3.5–5 mm and 3.2–4.5 mm long, pink to red or yellow near base; anthers oblong, dorsifixed, 1.5–2.4 mm long, 1–1.5 mm wide, purple. Style included or exserted beyond the tube, 6–9(–14) mm long, pale pink; stigma capitate, 0.4–1 mm thick, yellow-green or dull brown, or style acephalous and stigma non-functional. *Pistillate flowers* similar to staminate flowers, except: ovary 5–8 mm long. Floral tube 5.5–9 mm long, 3.8–5.4 mm wide at the rim. Sepals 4.1–6.4 mm long, 2.2–2.8 mm wide at the base. Filaments 1.6–2.7 mm and 1.6–2.4 mm long, red-purple; anthers sterile, 0.85–1.05 mm long, 0.4–0.7 mm wide, pink. Style exserted, 7–15 mm long; stigma capitate and 4-angled, 1.1–2.2 mm long, 1.8–2.5 mm wide, yellow-green; ovules 50–230 per ovary, biseriate in each locule. Berry ovoid-oblong to obovoid or subglobose, somewhat 4-angled, 10–25 mm long, 8–16 mm wide, bright red to magenta when ripe with a light purple, pruinous wax, fruiting pedicel 5–23 mm long; ovary wall firm, not becoming juicy or succulent at maturity, locules hollow; seeds tan, biseriate, oblong to (ob-)ovoid, 1.7–2.8 mm long, 1.3–1.6 mm thick, rugulose, usually between 20 and 80 per fruit. Gametic chromosome number $n = 11$.

Distribution (Fig. 6). A scarce plant in small populations along the far northern coast of the North Island of New Zealand, often associated with *Metrosideros excelsa* coastal forests or growing among *Phormium tenax* (flax) or *Cyperus ustulatus.* From North Cape to Maunganui Bluff on the west coast and to northern Coromandel Peninsula on the east coast, sea level to 150 m; flowering from October to March.

Representative specimens examined. NEW ZEALAND. **North Auckland:** North Cape District, 1896, (female), *Adams s.n.* (AK); Spirits Bay, Northland, Dec. 1926, (female), *Carse 982/A* (CHR); Kerr Point, 100 ft., 1 Sep. 1964, *G. K. R. & W. D. Burke s.n.* (WELTU); Scott Point, North Cape, Jan. 1913, *H. B. Matthews & Carse 982/4* (CHR); Houhora, base of Mount Camel at Cape Perpendicular, 6 Jan. 1952, (hermaphroditic-male), *Godley G2207* (CHR); Waihi, 20 mi. N of Ahipara, Jan. 1896, (male, reduced style), *Cheeseman s.n.* (AK, 2 sheets; WELT); near Reef Point, West Coast, Mangonui Co., Jan. 1901, *R. H. Matthews*, The Carse Herbarium No. 982/1 (CHR); Waipoua State Forest, Hokianga Co., 31 May 1983 (fr), *Wright 5600* (AK), 23 Feb. 1985, (male), *Wright 7094* (AK); S end of Maunganui Bluff, West Coast, growing among flax, 30 Nov. 1981, ("mainly male, few hermaphrodite"), *Lloyd s.n.* (CHR); Whangaroa, *Kirk s.n.* (WELT); Butterfly Bay, next to Tauranga Bay, in Whangaroa Bay, with *Ipomoea palmata*, 27 Mar. 1971, *Rawlings s.n.* (CHR); around the village of Matauri, on the East Coast, opposite the Cavallos Isles, inhabiting the sands immediately above the range of the tide, Mar. 1834, (female), *R. Cunningham* (K); Matauri Bay, 11 Dec. 1950, (male), *Godley G2203* (CHR, 2 sheets); Bay of Islands, Poraenui Point, with gorse and sheep under pohutukawa, 8 Nov. 1970, (male), *Rawlings s.n.* (CHR); Lizard Bay, Deep Water Cove, Bay of Islands, 16 Jan. 1970 (fr), *Engelhorn 25719* (CHR, 3 sheets), *25720* (CHR), Jan. 1965, (female), *Reynolds 155441* (CHR); Whangaruru, 18 Oct.

1969, (male), *Raven 25147* (CHR); Ngawae, 30 Dec. 1950, (female), *Godley G2206* (CHR, 2 sheets); Ngawae, Helena Bay area, 18 Oct. 1969, (male), *Raven 25145* (CHR); Ngahau Bay, Whangarei Co., Oct. 1946, (male), *Olsen N16/90–31* (AK); Matapouri, Dec. 1950, (male), *Godley G2209* (CHR, 2 sheets); Pataua River, Whangarei, Dec. 1876 (male), *Cheeseman s.n.* (AK); Bream Head, Whangarei, on shore of Peach Cove, 23 May 1979, *Ogle 96* (CHR). **Great Barrier Island:** Mine Bay, 21 Nov. 1950, *Trevarthen s.n.* (AK); Rangiwhakaea Bay, banks of Slip Stream, 1 Jan. 1983, (female), *Wright 5332* (AK); Whangaparapara Harbour to right of wharf, Nov. 1950, (female), *R. Lloyd 71615* (CHR); Tryphena Bay, 11 Dec. 1867, (male), *Kirk s.n.* (WELT 24809 and 24837); Johnson's Bay, directly S of Ruahine Trig., Apr. 1980, *Ogle 387* (CHR). **Coromandel Peninsula:** Cape Colville, Mellsop's Beach, 11 Dec. 1929, (female), *Moore 22139* (CHR); near Cabbage Bay, *Adams s.n.* (WELT); Rabart's Road, Tuateawa, 18 Oct. 1982, (male), *Smith-Dodsworth s.n.* (AK); Kennedy Bay, ca. 2 mi. along shore from N head of harbour, Jan. 1977, (male), *Bartlett N.40* (CHR).

In the summer of 1867 to 1868, Thomas Kirk collected at Great Barrier Island (Hamlin, 1965), where he gathered a procumbent plant which he "doubtfully" described as a variety of *F. procumbens* (Kirk, 1869: 147). However, duplicate material sent to Kew was described by Hooker (1871) as a new species, *F. kirkii*, based on differences in floral dimensions from *F. procumbens*. Soon thereafter, Hooker (1874) realized that the differences were due to sexual dimorphism, with *F. kirkii* based on the male morph of *F. procumbens*, and *F. procumbens* itself based on a female plant. In 1875, a second edition of volume 1 of the *Transactions and Proceedings of the New Zealand Institute* was printed (the original edition was printed in 1869). This second edition was paginated differently from the first one, so that Kirk's statement about the new variety of *F. procumbens* was on page 92 (Kirk, 1875). In this second edition, Kirk added the new name *F. kirkii* Hook. f. to the article, not yet aware that Hooker had changed his mind. This modified reprinting led Munz (1943) to give the following erroneous citation: "*Fuchsia kirkii* Hook. f. ex T. Kirk, Trans. and Proc. New Zealand Inst. 1: 92. 1868." Munz was obviously working from the second edition of Volume 1 and was either unaware that this was the second edition, or assumed that it was identical in content and pagination to the first. As a result, he gave the page number of the second edition (page 92) to the first edition (where it is page 147) and implied that the name *F. kirkii* is present in the first edition, when it is not. He also miscited the date of the first edition as 1868, rather than 1869.

Fuchsia section **Skinnera** (J. R. & G. Forster) de Candolle, Prodr. 3: 39. 1828. *Skinnera* J. R. & G. Forster, Char. Gen. Pl. 58, pl. 29. 1776. TYPE: *Fuchsia excorticata* (J. R. & G. Forster) Linnaeus f.

Trees, shrubs, or lianas. Leaves alternate to opposite or rarely ternate, membranous to coriaceous, entire to sinuate or serrulate, weakly to strongly bicolored, light green to white or silvery on lower surface, the blade subequal to or longer than the petiole. Hermaphroditic or gynodioecious; flowers axillary and solitary, pendulous or divergent, sometimes grouped on shortened side shoots, females smaller than hermaphrodites. Floral tube cylindrical or obconical with a sharp constriction above a bulbous base, green in bud, initially green at anthesis but then changing to red or purple in late anthesis; nectary a smooth band lining the inner, basal part of the tube. Sepals spreading or recurved. Petals lanceolate, erect, 1.2–11 mm long, purple. Stamens biseriate, the antesepalous stamens longer than the antepetalous ones; pollen bright blue, 2-aperturate, with beaded to nearly smooth viscin threads. Stigma capitate to quadrangular, slightly 4-lobed when functional. Ovary with ca. 500–700 multiseriate ovules. Berry oblong, 5–16 mm long, red to deep purple, fleshy; seeds oblong-triangular to oblong, 0.75–1.2 mm long, 0.3–0.6 mm wide. Gametic chromosome number n = 11.

Distribution (Figs. 5 and 6). New Zealand and Tahiti (Society Islands).

2. Fuchsia cyrtandroides J. W. Moore, Occas. Pap. Bernice Pauahi Bishop Mus. 16(1): 13, fig. 8. 1940. TYPE: French Polynesia. Society Islands: Tahiti, S side of Mount Orohena, rainforest, 1500 m, occasional tree 5 m high, 15 cm diam., 14 May 1927, *L. H. MacDaniels 1315* (holotype, BH, photograph, POM; isotype, BISH, fragment at POM 260882). Figure 36.

Small tree (2–)3–7 m tall, with numerous ascending branches; trunk with tan-copper, slightly fissured bark, 5–30 cm diam. Branchlets subglabrous, 4–6 mm thick, usually quadrangular. Leaves mostly opposite, occasionally ternate, less commonly alternate or subopposite, subcoriaceous to firm-fleshy, (broadly) elliptic or obovate, (2–)4–12 cm long, (1.5–)2–6 cm wide, rounded or obtuse at apex, rounded to acute at base, green above, whitish below and sometimes purple-flushed; margin subentire with small, reddish, glandular teeth; midvein raised below, secondary veins (7)8–12 on each side. Petioles stout, 1.5–3 mm thick when fresh, 5–

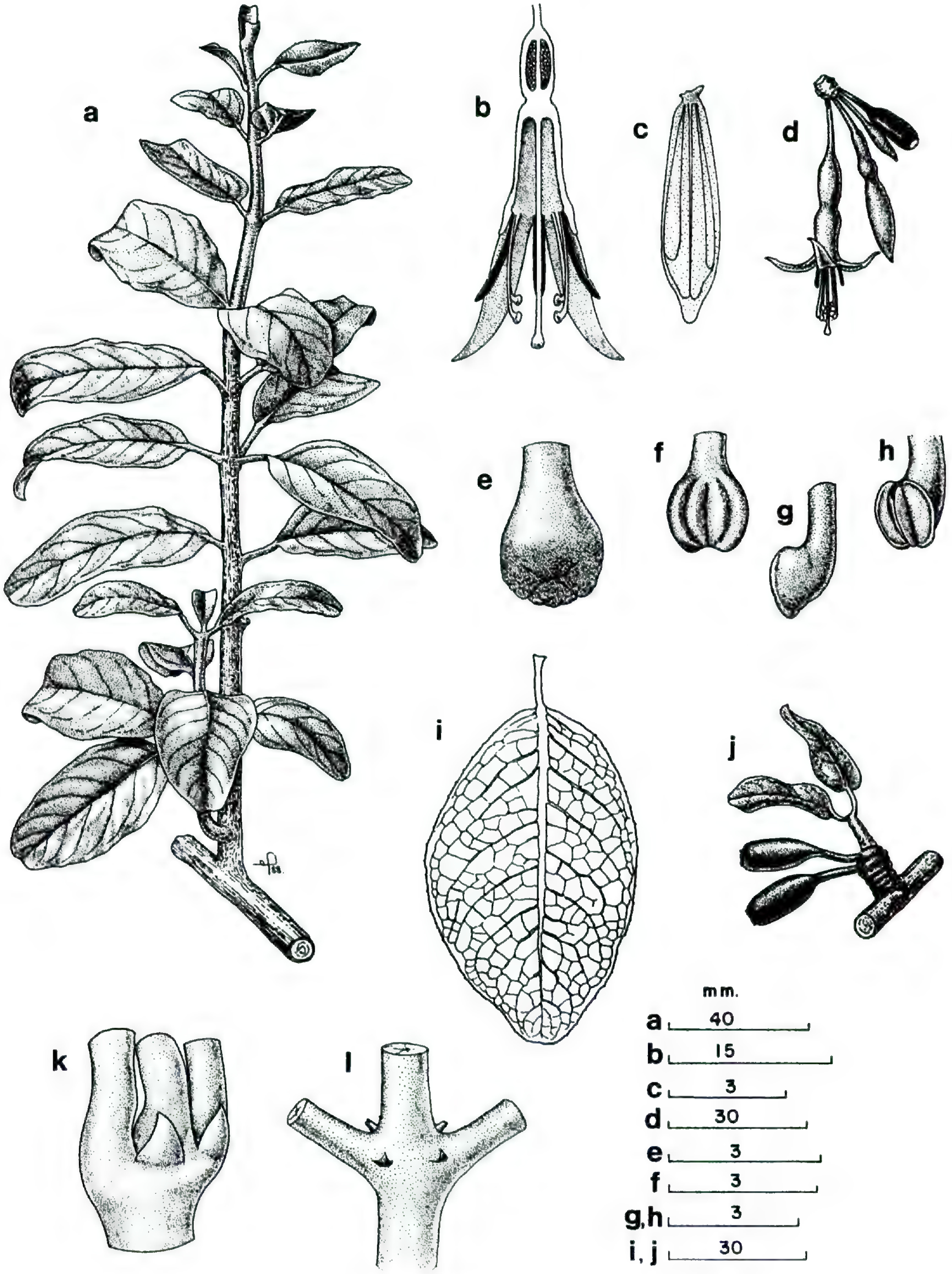


FIGURE 36. *Fuchsia cyrtandroides.*—a. Habit.—b. Longitudinal section through a flower.—c. Petal.—d. Ramiflorous cluster of flowers.—e. Stigma.—f. Anther, before dehiscence, facing toward the central style.—g. Side view of anther.—h. Dehiscent anther.—i. Adult leaf showing venation pattern.—j. Young fruits on a side shoot with young leaves emerging.—k. Young leaf node showing stipules.—l. Older leaf node, as the stem enlarges and the stipules begin to dry out. Drawing by E. Pérez.

25(–35) mm long. Stipules triangular, dark purple, 0.5–0.8 mm long, 0.40–0.75 mm wide and crassate at the base, caducous.

Hermaphroditic; flowers axillary on young, leafy shoots or grouped on short, leafless side shoots off the main branches. Pedicels stout, 5–20 mm long, pendulous in flower, divergent to erect in fruit, with the upper 5 mm enlarged and dark purple. Ovary cylindrical-fusiform, 6–8.5 mm long, 3–4.5 mm thick, green; ovules 500–600, 4- or 5-seriate in each locule. Floral tube cylindrical to slightly fusiform, slightly tetragonous, 6–14 mm long, 5–9 mm wide at the rim; nectary poorly defined, 3–5 mm high. Sepals lanceolate, 7–19 mm long, 3.5–7 mm wide at the base, spreading to recurved. Floral tube and sepals green with dark purple streaks in bud and at anthesis, changing abruptly to dark crimson-purple before dehiscing. Petals oblanceolate to elliptic, 4–11 mm long, 1–4 mm wide, erect, lavender to light purple, turning darker with age. Filaments 0.8–1 mm thick, 6–18 mm and 5–12 mm long, purple; anthers suborbicular, attached apically with a thick connective, 1.3–1.8 mm long, 1.5–2.5 mm wide, purplish, with reniform thecae. Style stout, 1–1.5 mm thick, 14–30 mm long, faint cream-colored to purple; stigma clavate to capitate, 1.5–3 mm long, 1.5–3 mm wide, faintly 4-cleft at apex, light green. Berry oblong-fusiform or ellipsoid, 9–16 mm long, 5–10 mm wide, purple-black and juicy when ripe; seeds oblong-triangular, 0.9–1.2 mm long, 0.4–0.7 mm thick, light brown. Gametic chromosome number $n = 11$.

Distribution (Fig. 5). Endemic to several of the highest peaks on the western (drier) side of the island of Tahiti, from 1150 to 2000 m; flowering mostly in dry season (July–October), but occasionally and less intensively in other months throughout the year.

Specimens examined. FRENCH POLYNESIA. TAHITI. **Mont Marau:** along road to antenna and in adjacent valleys, 1200–1450 m, 4 Feb. 1988, *Berry, Guérin & Florence 4628* (PAP, MO); N flank of Mont Marau, roadside, 1370 m, 24 Mar. 1982, *Florence & Guérin 2713* (PAP, 2 sheets); Haute Tipaerui, sentier *Fuchsia*, 1380 m, 26 July 1983, *Florence 4700* (BISH, K, NY, P, PAP, US), 15 Oct. 1982, *Flynn & Flynn 329* (PAP); highest slopes and ridges around the disturbed summit, 1480–1490 m, 19 July 1981, *Fosberg 61095* (CHR); Mont Marau road, crest between Tapaerui and Punaruu valleys, 1250 m, 5 June 1982, *Fosberg 62646* (CHR); 1 Sep. 1973, *Godley* (CHR); 1400 m, 20 Mar. 1977, *Sachet 2352* (MO, US), 2 Sep. 1980, *Veillon & Guérin 38* (BISH, P, PAP, US). **Mont Aorai:** "Les Grosses Pierres," 4000 ft., 15 June 1962, *Carlquist 653* (DS, 2 sheets, RSA; $n = 11$); summit crests above Fare Ata, Comune de Pirae, 1900 m, 8 Apr. 1986, *Florence 7631* (BISH, K, P, PAP); right side of the high valley of Pirae, below the Aorai path, Comune de Arue, 1405 m, 18 July 1986, *Florence 7748* (PAP); 3 Sep. 1973, *Godley* (CHR); Sentier Aorai, crêtes bord ravin, 1500 m, 10 July 1964, *Maclet 156* (CHR); Aorai, Comune de Mahina, 4000 ft., 5 June 1930, *Grant 3742* (A, BISH, K). **Mont Orohena:** N flank of summit of Mt. Pitohiti, path to Orohena, Comune de Mahina, 2000 m, 19 Oct. 1983, *Florence 5455* (BISH, P, PAP, US); S side of Mont Orohena, 1500 m, 16 May 1927, *MacDaniels 1341* (BISH); S ridge, 1600 m, 22 Sep. 1934, *St. John & Fosberg 17005* (BISH).

3. **Fuchsia perscandens** Cockayne & Allan, Trans. Proc. New Zealand Inst. 57: 53, fig. 1. 1926. TYPE: New Zealand. North Island: Kitchener Park, Feilding, forest margins as liane, Sep. 1926, *H. H. Allan 982a* (lectotype, selected here, CHR 330298; see Fig. 37).

Slender and densely branched, usually decumbent shrub 1–2 m tall, or liana 2–8 m tall with strongly flexuous stems to 5 cm diam. and up to 10 m long; bark brown, flaking off in strips; branchlets strigulose. Leaves alternate, firm-membranous, broadly ovate to suborbicular, 7–40 mm long, 5–33 mm wide, acute to rounded at apex, subcordate to rounded at base, pale green, paler beneath, glabrous to strigulose along the margins and veins; margin remotely serrulate to sinuate; secondary veins 3–5 on either side. Petiole slender-filiform, semi-translucent, 10–40 mm long, subequal to the blade length, strigulose above, the basal 1–1.5 mm swollen and crassate, purple when fresh, tan when dry, generally persistent on the stem after the leaf dehisces. Stipules narrowly triangular, 0.8–1.3 mm long, 0.3–0.6 mm wide, semisucculent and purple when young, caducous.

Gynodioecious. *Perfect flowers*: solitary, sparse; pedicels slender, drooping, 4.5–12.5 mm long. Ovary cylindrical, 3–7 mm long, 1–2.4 mm thick, green. Floral tube 9–16 mm long, 2.2–4 mm wide and bulbous at the base, lined by a smooth nectariferous band inside, sharply constricted above to 1.3–2 mm wide, then widening abruptly to 5.5–7.5 mm wide at the rim, 4-angled in bud. Sepals lanceolate, 7–12 mm long, 3–4.4 mm wide at the base, spreading to occasionally reflexed. Tube and sepals lustrous green at anthesis, with dull purple streaks in upper parts between the ridges of the tube, both changing ± abruptly to red-crimson throughout before dehiscing. Petals dark purple, elliptic, 1.5–4 mm long, 0.6–1 mm wide in the middle, acute or obtuse at apex. Filaments 5.2–11 mm and 4.2–8 mm long, purple, pale cream on connective; anthers 1.6–2.5 mm long, 1–1.7 mm wide, purple. Style 20–36 mm long, pale cream to purple; stigma capitate, 1–1.9 mm long, 0.8–1.1 mm wide, yellow-green. Berry subcylindric, 5–9 mm long, 4–5.5 mm

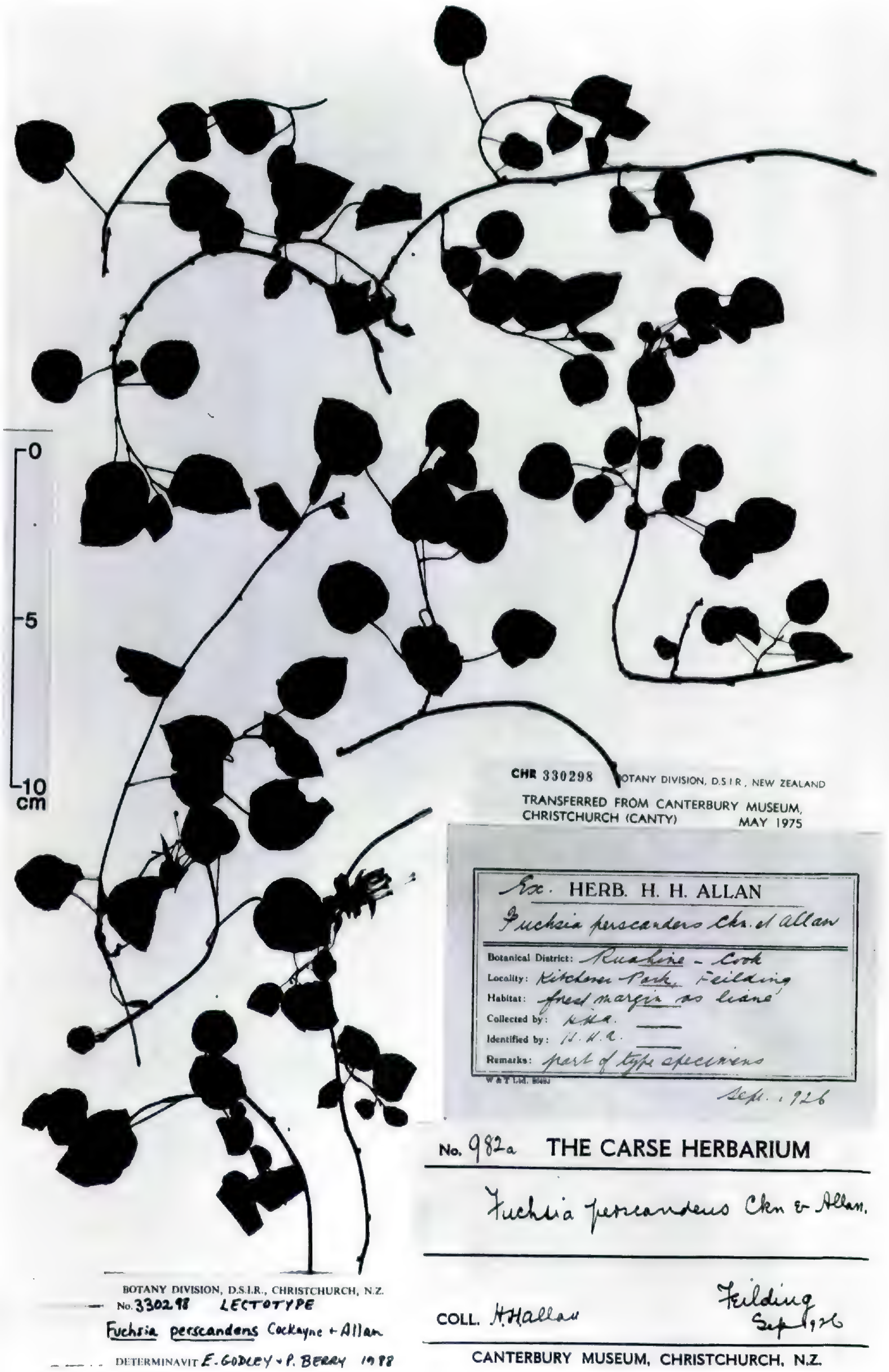


FIGURE 37. Lectotype of *Fuchsia perscandens*, CHR 330298. Photograph by R. Lamberts.

thick, dark purple when ripe; seeds oblong-triangular, 0.8–1.1 mm long, 0.4–0.6 mm wide. *Pistillate flowers*: similar to perfect ones except: pedicels 4–16 mm long. Floral tube 5.8–10.8 mm long, 1.1–2.5 mm diam. at the constriction, 3.5–5.3 mm wide at rim. Sepals 5.7–9 mm long, 2.6–3.4 mm wide at base. Filaments 2.5–5.2 and 1.5–4.4 mm long; anthers aborted, 0.7–1.1 mm long, 0.5–0.7 mm thick. Style 16–27 mm long; stigma 1–2.2 mm thick. Gametic chromosome number $n = 11$.

Distribution (Fig. 6). New Zealand, infrequent in lowland forests, especially at margins and near clearings, on the North Island as far north as 36°S latitude (historical data), now known no farther north than 37°15′S latitude near Paeroa, and throughout the South Island at scattered localities as far south as Bluff (46°36′S), 0–550 m; flowering from August to March.

Representative specimens examined. NEW ZEALAND. **Northern North Island:** Northern Wairoa, *Cheeseman in Jan. 1875* (AK, 2 sheets); near Whangape Lake, Lower Waikato, *Kirk s.n.* (WELT); Opuatia Creek, Waikato, *Kirk s.n.* (WELT); Whangamarino Swamp, near Kopuku coalmine, *Ogle 563* (CHR), *726* (CHR); 8 mi. NW of Paeroa, *Bartlett TV181* (CHR); Thames River, Te Aroha, *Cheeseman in Jan. 1884* (AK); Hammond Bush, Hamilton City, *de Lange on 4 Dec. 1991* (AK); White Pine Bush, near Whakatane, *Hynes in 23 Dec. 1966* (AK); Otorohanga Co., Awaroa-Mahoenui Valley Road, Awaroa Scenic Reserve, near Te Koraha, *de Lange in 21 Oct. 1984* (AK); Aranui Cave, Waitomo Caverns, *Raven 25192–2* (CHR), *25192–4* (CHR); Waitomo Co., Mangapu Stream, 5 km NW of Te Kuiti, *Gardner 3142* (AK, WELT). **Central North Island:** Taumarunui, *Davidson & Petrie in Nov. 1908* (WELT); Taumarumi Co., Kouturoa Stream, 2 km S of Kaitieke, *Gardner H164* (AK); Ngawaka, N of Taihape, *Druce in Oct. 1978* (CHR); Mataroa, NW of Taihape, *Druce in Feb. 1979* (CHR); Potaka Bush, Rangitkei Valley, SE of Taihape, *Druce in Nov. 1979* (CHR); Kawhatau Valley, E of Mangaweka, *Druce in Apr. 1977* (CHR); NE of Hunterville, *Druce in Dec. 1978* (CHR). **Eastern North Island:** Hangaroa Valley, W of Whakapunake, *Druce in Dec. 1967* (CHR); Waipipapoa, S of Havelock North, *Druce in Oct. 1973* (CHR); Elsthorpe Domain, Hawke Bay, *Druce in Apr. 1980* (CHR), *Elder 723/2* (CHR); Waipukurau Scenic Reserve, Hawke Bay, *Druce in Nov. 1975* (CHR); Dannevirke, *Colenso s.n.* (WELT 22767, mixed collection with *F.* ×*colensoi*); Mangapuaka Stream, E of Dannevirke, Hawkes Bay, *Druce in June 1982* (CHR). **Western North Island**: Marokopa River, W of Te Kuiti, *Druce in Oct. 1978* (CHR); Tututawa, 12 mi. E of Stratford, *Hedditch in Nov. 1966* (CHR); Gordon's Park Reserve, near Wanganui, *Raven 25132* (CHR); Okura Banks, Wanganui River, *Phillips s.n.* (AK). **Southern North Island:** E of Masterton, Wairarapa Dist., 1.5 mi. W of Carswell, *Macmillan 70/269* (CHR); Maungaraki River, Wairarapa, *Druce in Jan. 1966* (CHR); Tauweru Valley, E of Carterton, Wairarapa, *Druce in Mar. 1976* (CHR); Carter's Bush, near Ruamahanga River, *Raven 25121* (CHR, 7 sheets); Merry Hill Farm, Feilding, *Allan 173* (CHR); Kitchener Park, near Feilding, *Healy in 3 Oct. 1934* (CHR, WELT); Pohangina Valley, *Druce in Oct. 1973* (CHR); Bledisloe Park, Massey, *Raven 25123* (CHR, 3 sheets); Tiritea Stream, Fitzherbert West, *Healy s.n.* (CHR); Keeble's Bush, S of Palmerston North, *Druce in Feb. 1979* (CHR); Himatangi Bush, on Barber Estate, *Raven 25127* (CHR, 10 sheets); 0.5 mi. W of Ohau, *Duguid in 18 Oct. 1953* (CHR); Otaki Gorge, *Atkinson in 20 Feb. 1931* (WELT); Pakuratahi River, near track to Eastern Hutt, *Mason in 1 Dec. 1945* (CHR); Silverstream Bush, Hutt Valley, *Druce in Jan. 1973* (CHR); stream W of Sinclair Head, S Wellington Coast, *Ogle 434* (WELT); near Cape Terawhiti, *Healy 1023* (CHR); Red Rock Stream, 0.5 mi. from coast W of Wellington, *Raven 25206–1* (CHR). **Northern South Island:** mouth of Kohaihai River, 10 mi. N of Karamea, NW Nelson, *Macmillan 69/56* (CHR); Kahurangi Point, NW Nelson, *Collett in Mar.–Apr. 1965* (CHR); Kaihoka Point, NW Nelson, *Druce in Dec. 1978* (CHR); Motueka, *Healy in 27 Feb. 1944* (CHR); along D'Urville River near Lake Rotoroa, *Raven 25567* (CHR, 2 sheets); Canvastown, Pelorus, *Kirk in 22 Nov. 1886* (WELT); E of Taylor's Pass, S of Blenheim, Marlborough, *Druce in Nov. 1976* (CHR). **Eastern South Island:** Ngawiro, Waiau, *Simpson 8251* (CHR); hills E of Parnassus, *Robins & Mitchell in 6 Jan. 1969* (CHR); Ethelton, Hurunui River, *Healy in 20 Dec. 1943* (CHR); Lower Waipara, *Oliver in 29 Aug. 1948* (WELT); Kowai Bush, railway station between Springfield and Arthur's Pass, *Talbot in 1947* (CHR); Selwyn Gorge, *Oliver in 23 Nov. 1947* (CHR, WELT); Riccarton Bush, Christchurch, *Raven 25212* (CHR, 8 sheets); Summit Road, Kennedy's Bush, Sign of the Bellbird, *Berry & Brako 4622* (CHR); Stony Bay, N of Okain's Bay, Banks Peninsula, *Ericson in 9 Dec. 1971* (CHR); Rakaia Gorge, 8 mi. from Lake Coleridge, opp. Mt. Hutt range, *Talbot in Jan. 1949* (CHR); Mount Peel, *Allan 394* (CHR); King's Gully, Timaru, *Mason in 11 Nov. 1945* (CHR, 2 sheets); Hunters Hills, Hendry's Bush, *Macmillan & Woodhouse 72/1200* (CHR). **Western South Island:** Karamea, *Moore in 26 Dec. 1957* (CHR); 2 mi. E of Te Namu, S of Karamea, *Raven 25239* (CHR, 10 sheets); Westport Domain, SW Nelson, *Kelly & Kelly in 10 Oct. 1973* (CHR); Cape Foulwind, Westport, *Berry & Brako 4598* (CHR); Punakaiki, *Raven 25224* (CHR, 7 sheets); Paroa, S of Greymouth, *Oliver in 17 Apr. 1948* (CHR, WELT); Arnold River Scenic Reserve, Moana, *Loh in 21 Nov. 1978* (CHR); mouth of Mikonui River, *Wardle & Myers in 3 Dec. 1969* (CHR); 1 mi. N of crossing of Paringa River, S Westland, *Raven 25346* (CHR); N side of Cascade River Mouth, S Westland, *Wardle in 31 Mar. 1977* (CHR). **Southern South Island:** Pars Creek, NE of Dansey's Pass, N Otago, *Wardle in 5 Jan. 1975* (CHR); N of Hampden, E Otago, *Macmillan 72/919* (CHR); Shag River Valley, 5 mi. NW of Dunback on Highway 85, *Meurk in 24 Sep. 1971* (CHR); Otago Peninsula, Broad Bay, *Johnson in 20 Oct. 1981* (CHR); Kaitangata, E Otago, *Petrie in Oct. 1892* (WELT); Kelso, near Tapanui, Otago, *Petrie in Dec. 1886* (WELT, mixed collection with *F.* ×*colensoi*); 1.5 mi. N of Lumsden, central Otago, *Atwood in Jan. 1940* (AK); Coal Creek Rock Bluff, Umbrella Ecological District, Otago, *Dickinson & Mark in 24 Jan. 1986* (CHR, OTA); Bluff Head, S coast, *Oliver in 12-X-1945* (WELT); Oreti River, Invercargill, *Holloway s.n.* (OTA).

In the protologue to *Fuchsia perscandens*, Allan stated that his description was based on living plants growing at Kitchener Park. The lectotype here designated was annotated by Allan in September 1926 as "part of type specimens" of *F. perscandens*, and is clearly one of the plants used to

describe this species. When Allan's treatment for the Flora of New Zealand was published (Allan, 1961: 282), however, he designated an undated specimen from Kitchener Park (*Allan s.n.*, CHR 11479) as the lectotype. This choice is superseded because the specimen is a hybrid between *F. perscandens* and *F. excorticata* and is in conflict with the protologue (see also Allan, 1927, for a discussion of the hybrids at Kitchener Park). Allan did not have access to all of his original specimens when he prepared his flora, and he apparently did not consult *Allan 982a*, which then formed part of the Carse Herbarium (CANTY).

4. **Fuchsia excorticata** (J. R. & G. Forster) Linnaeus f., Suppl. Pl. 217. 1781. *Skinnera excorticata* J. R. & G. Forster, Char. Gen. Pl. 58, pl. 29. 1776. TYPE: New Zealand. Queen Charlotte Sound: either 2–25 Nov. 1773 or 19 Oct.–10 Nov. 1774, *G. Forster s.n.* (lectotype, selected here, Herb. Smith 670.7, LINN).

Large shrub or tree 2–13 m tall, winter deciduous in most of its range; trunk generally irregular, ± twisted, to 70 cm diam., branching within several m of the base to form a broad, spreading canopy; bark coppery light brown and papery, peeling off readily in patches or large strips, with a smooth, green cambial layer beneath the outer layers; branches subglabrous to strigulose. Leaves alternate, thick-membranous, lanceolate to ovate-elliptic, (35–)45–130 mm long, (15–)25–55 mm wide, acute to acuminate at apex, acute to rounded at base, strongly bicolored, medium to dark green above, silvery white below or occasionally suffused with red, glabrous to strigulose along the margins and veins beneath; margin entire to remotely serrulate; secondary veins 9–12 per side. Petiole 9–40 mm long, subglabrous to strigulose above. Stipules lanceolate, purplish, 1.5–4 mm long, 0.3–0.8 mm wide, drying out from the tip down, caducous.

Gynodioecious. *Perfect flowers*: solitary or ± tightly grouped on shortened side shoots, sometimes appearing cauliflorous; pedicels slender, drooping in bud and flower, divergent or ascending in fruit, 5.5–15 mm long. Ovary 4.5–8 mm long, 1.7–3 mm thick, green. Floral tube 11–22 mm long, 3–5 mm wide at the bulbous base (lined by smooth nectariferous tissue inside), sharply constricted above to 1.4–2.5 mm diam., then enlarged until 4–10 mm wide at the rim, with 4 antepetalous ridges. Sepals narrow-triangular, 8–19 mm long, 3–6 mm wide at base, spreading or occasionally reflexed. Tube and sepals lustrous green at anthesis, with dull purple streaks in upper parts between the ridges of the tube, both changing ± abruptly to red-crimson about halfway through anthesis. Petals dark purple, (narrowly) elliptic or lanceolate, 1.8–5 mm long, 0.7–2.1 mm wide in the middle, acute or obtuse at apex. Filaments 6–15 mm and 5–10 mm long, light to dark purple, pale cream on connective to anther; anthers 1.8–2.5 mm long, 1–1.7 mm thick, purple. Style glabrous, 22–40 mm long, purple in exserted part and greenish below; stigma capitate, 1.8–2.2 mm long, 1.4–2.1 mm wide, yellow-green. Berry oblong-ellipsoid, 9–13 mm long, 5–8 mm thick, dark purple; seeds oblong-triangular, 0.75–1 mm long, 0.3–0.6 mm broad, tan, 320–670 (mostly around 500) per fruit. *Pistillate flowers*: similar to perfect ones except: pedicels 4.5–8.5 mm long. Ovary 4–6 mm long, 2–2.5 mm wide. Floral tube 6.5–13 mm long, 4–6 mm diam. at rim. Sepals 5.5–8 mm long, 2.2–3.5 mm wide at base. Petals 1.2–2.6 mm long, 0.35–1 mm wide. Filaments 2–4 mm and 1.2–3.5 mm long; anthers aborted, 0.8–1.2 mm long, 0.6–0.9 mm wide. Style 18–23 mm long. Gametic chromosome number $n = 11$.

Common names. kotukutuku (Maori, for whole plant), konini (Maori, for the berry), tree fuchsia, native fuchsia.

Distribution. Widespread all over North Island, South Island, and Stewart Island of New Zealand, rare on Auckland and Chatham Islands, in lowland to upper montane forest, especially along margins and near streams, 0–1050 m; flowering mostly from August to December, but occasionally from as early as June or July and as late as the end of January.

Representative specimens examined. NEW ZEALAND. **Northern North Island:** Mangonui Co., Te Paki Coastal Park, 'Radar Bush', *Bartlett s.n.* (CHR 314556); Mangonui, Kaitaia, *Matthews 2311* (AK); Cavalli Islands, Motukawanui Island, ¾ way up Waiti Stream, *Court s.n.* (AK 149877); Whangarei Co., Pikiwahine-Tauraroa Rd., *Cooper s.n.* (AK 120225); Hen & Chickens Island, Hen Island, above Dragon's Mouth Cove, *Wright 2226* (AK); Little Barrier Island, *Shakespear s.n.* (AK 127539); Hobson Co., Katui, *Cooper s.n.* (AK 127370); Titirangi, near Auckland, *Carse 980/4* (CHR); Mt. Wellington lava fields, Auckland, *Petrie in Sep. 1896* (WELT); Mauku, Manukau Co., *Carse 980/8* (CHR); Hauraki Gulf, Ponui (Chamberlin's) Island, *Brown s.n.* (AK 151199); Great Barrier Island, east coast scrub-bush, N of Whangapoua Beach, *Frater, Cooper & Stephenson 355* (AK); Coromandel Co., Mercury Bay, *Wright 633* (AK); Tirau, upper Thames valley, *Petrie in Nov. 1894* (WELT); Tauranga-Hamilton old road, Kaimai Range, *Chapman in 2 Oct. 1973* (CHR); Waioeka Gorge, Opotiki Distr., *Heginbotham in Aug. 1975* (CHR); 4 mi. E of Rotoma Saddle on Rotorua-Whakatane road, *Hamilton in 3 Nov. 1970* (CHR); Lake Tarawera, *Lambrechtsen in 12 Jan. 1971* (CHR); 7 mi. N of junction near Kawhia, road to Raglan, *Raven 25181* (CHR); 6 mi. E of Waitomo, *Raven 25187* (CHR). **Central North Island:** Maraeroa, 17 mi. SE of Mangapehi, *Rawson in 20*

Jan. 1947 (CHR); Taumarunui, *Davidson in 1909* (WELT); Mt. Pihanga, Taupo, *Parsons 225* (CHR); W slopes of Mt. Tongariro, *Atkinson in 12 Jan. 1964* (CHR); Three Kings Range, Kaimanawa Range, *Druce in Jan. 1974* (CHR); 7 mi. S of Raetihi, *Raven 25134* (CHR, 2 sheets). **Eastern North Island:** Mt. Hikurangi, Raukumara Range, Gisborne Prov., *Veitch s.n.* (CHR 191554); Pakihiroa, *Oliver in 16 Nov. 1926* (WELT); Huiarau Range, highest point, *Lambrechtsen in 11 Jan. 1971* (CHR); N of Te Haroto, Hawke Bay, *Macmillan 72/1021* (CHR); Maraetotara Valley, S of Havelock North, *Druce in Oct. 1973* (CHR). **Western North Island:** 0.5 mi. below Marokopa Falls, near Te Anga, *Lambrechtsen in 14 Jan. 1971* (CHR); Dawson Falls, Mt. Egmont, *Heine in 29 Dec. 1932* (WELT); Opunake, *Druce in Nov. 1970* (CHR); Tokomaru E Road, Wanganui, *Wanganui Museum Botanical Group 40* (CHR). **Southern North Island:** Ruahine-Cook, Rangitikei Gorge, *Zotov 193* (CHR); Alfredton, Wairarapa District, E of Eketahuna, *Macmillan 70/277* (CHR); Heatherlea East, N of Levin, *Duguid s.n.* (CHR 194271); Bannister's Hut, Mt. Holdsworth, *Heine in 14 Jan. 1930* (WELT); Kahoa Stream, Pukerua Bay, *Ogle in 12 Sep. 1974* (WELT); Park Vale, Karori, *Kirk in 8 Nov. 1883* (WELT, 3 sheets); Palliser, *Healy s.n.* (WELT); Haurangi Mts., *Oliver in 7 Dec. 1930* (WELT, 3 sheets). **Northern South Island:** bluff behind cottage SW of Kaihoka Lakes, *Given 69879* (CHR); Karamea, in carpark at Oparara Arches, *Manning in 15 Dec. 1984* (CHR); Riwaka River, N.W. Nelson, 300 ft., *Druce in Nov. 1974* (CHR); 5.5 mi. W of Murchison, *Godley in 3 Feb. 1971* (CHR); Capleston Reserve, N of Reefton, *Macmillan in 25 Jan. 1972* (CHR); near Maruia Springs, 800 m, *Berry & Brako 4596* (CHR); Tennyson Inlet, Marlborough Sounds, *Hynes in 16 Feb. 1969* (CHR); Canvastown, Pelorus, *Kirk in 22 Nov. 1886* (WELT); Shin Creek, inland Kaikoura, *Hamlin 919* (WELT); Lynton Downs, Kaikoura, *Kirk in Dec. 1889* (WELT). **Eastern South Island:** Lewis Pass Rd., near junction of Doubtful and Boyle Rivers, *Godley in 3 Feb. 1971* (CHR); upper Hurunui River, 2 mi. W of Lake Sumner, *Macmillan & Stemmer in 29 Jan. 1973* (CHR); Motunau River, N Canterbury, *Mason & Simpson in 22 Nov. 1960* (CHR); View Hill Reserve, Oxford, *Moore in 11 Oct. 1969* (CHR); Woodstock, *Moore in 3 Jan. 1971* (CHR); Riccarton Bush, Christchurch, *Raven et al. 25214* (CHR); near Cass Peak, Port Hills, *Macmillan in July 1964* (CHR); head of Kaituna Valley, Banks Peninsula, *Kelly in 18 Nov. 1965* (CHR); NW of Staveley, Ashburton County, *Macmillan in 30 Sep. 1970* (CHR); Peel Forest, South Canterbury, *Rawson in 2 Oct. 1974* (CHR); Bluecliffs Station, Hunters Hills, South Canterbury, *Sykes in 14 Apr. 1971* (CHR). **Western South Island:** Punakaiki, *Raven 25222* (CHR); 1 mi. S of Barrytown, *Raven 25219* (CHR); Crushington, near Reefton, *Molloy in 4 Mar. 1988* (CHR); Omotomotu Scenic Reserve, *Loh in 16 Nov. 1978* (CHR); Otira Gorge, *Raven 25334* (CHR); E of Kumara, *Berry & Brako 4615* (CHR); near Hokitika, *Woods in 29 Nov. 1987* (CHR); foot of Mt. Hercules, *Wardle in 30 Nov. 1970* (CHR); Lake Wahapo, *Wardle in 30 Nov. 1970* (CHR); valley just below Franz Josef Glacier, *Raven 25337* (CHR); Bruce Bay, south Westland, *Wardle in 7 Jan. 1977* (CHR); Haast River, at Thundering Falls, *Raven 25358* (CHR). **Southern South Island:** Leith Valley, Dunedin, *Oliver in Dec. 1940* (WELT); Hoopers Inlet, Otago Peninsula, *Johnson in 17 Oct. 1971* (OTA); Tautuku Reserve, South Otago, *Esler in Jan. 1978* (OTA); Dunsdale, Hokonui Hills, Southland, *Miller in 17 Apr. 1976* (OTA); Luggate Creek, Pisa Range, 1100 ft., *Mark & Wells in 1 Dec. 1966* (OTA); Lake Wakatipu shore, *Oliver in 11 Feb. 1953* (WELT); Falls Creek, upper Hollyford valley, *Salmon in Dec. 1943* (WELT); Milford side of Homer Tunnel, Fiordland, *Enting in 13 Apr. 1973* (WELT); Divide Creek, N of Lake Fergus, *Raven 25431* (CHR); head of Stillwater River, Fiordland, *Anderson in 2 Jan. 1971* (CHR); Lake Shirley, Fiordland, *Given 69543* (CHR); Lake Manapouri, head of South Arm, *Raven 25420* (CHR); Deep Cove, Doubtful Sound, *Macmillan in 12 Dec. 1962* (CHR); Hut Creek, Supper Cove, Dusky Sound, Fiordland, *Given 72545* (CHR); Wet Jacket Arm, valley S of Mt. Anderson, Fiordland, *Given 72207* (CHR). **Stewart Island:** N Arm, Port Pegasus, *Ritchie in 22 Feb. 1966* (CHR); Deep Bay, Paterson Inlet, *Wilson 789–85* (CHR); Half-Moon Bay, *Brownlie 84* (CHR); Ernest Island off Port Pegasus, *Yaldwyn in 1 Mar. 1972* (WELT); road to Fern Gully, *Goulding 761* (AK), *762* (AK). **Chatham Island:** S of Lake Huro, *Hamel in 28 Jan. 1976* (CHR, OTA); Te Awatea, S shore of Lake Huro, *Given & Williams 13023* (CHR); by Lake Huro, *Ritchie & Ritchie in 16 Sep. 1968* (CHR); Maipito Bush, Waitangi, *Hamel in 29 Jan. 1976* (OTA). **Auckland Island:** Auckland Is., *Aston in Jan. 1909* (AK, WELT); Port Ross, *Aston s.n.* (WELT); Laurie Harbour, *Dawbin in 25 Aug. 1943* (CHR), *Oliver in 27 Mar. 1927* (WELT); mouth of Grey Duck Stream, *Godley in 29 Dec. 1962* (CHR).

5. Fuchsia ×colensoi Hooker f. (pro sp.), Handbk. N. Z. Fl. 728. 1867. TYPE: New Zealand. North Island: from woods at Titiokura, *William Colenso 1048* (lectotype, selected here, K; Fig. 38).

Fuchsia ×experscandens Allan, Genetica 9: 507, fig. 5. 1927. TYPE: New Zealand. North Island: forest remnants near Feilding, Merry Hill Farm, Feilding, *H. H. Allan in Sep. 1926* (lectotype, selected here, CHR 11523). There are six sheets numbered 11519–11524 at CHR, and the lectotype is from a female plant.

Small to large shrubs or small trees, the main branches upright, scandent-climbing or decumbent; main stem to 30 cm diam.; bark papery, copper-colored, readily flaking off; branchlets subglabrous to strigulose. Leaves alternate, thick-membranous, usually broadly elliptic-ovate, lance-ovate or suborbicular, 18–85 mm long, 10–45 mm wide, obtuse to acute at apex, truncate to obtuse or subcordate at base, pale green above, paler green to whitish beneath, glabrous to strigulose along the margins and veins beneath; margin entire to serrulate with small, glandular teeth; secondary veins (5)6–8(9) per side. Petiole slender, 5–50 mm long, subglabrous to strigulose above, sometimes with a semi-persistent swollen base. Stipules triangular, 0.8–2.4 mm long, 0.4–1.1 mm wide, semisucculent and purple when young, quickly drying from the tip, caducous.

Gynodioecious, the flowers intermediate in size between *F. excorticata* and *F. perscandens*. *Perfect flowers*: pedicels slender, hanging, 5–14 mm long.

FIGURE 38. Lectotype of *F.* ×*colensoi*. Photograph courtesy of the Royal Botanic Gardens, Kew.

Ovary 4–7 mm long, 1.6–2.5 mm thick, green. Floral tube 9–20 mm long, 2.5–4.2 mm wide and bulbous at the base, constricted above to 1.2–2.3 mm wide, then ampliate until 4–9 mm wide at the rim. Sepals narrow-triangular, 7–14 mm long, 3.5–5 mm wide at base, spreading. Tube and sepals lustrous green at anthesis with dull purple streaks in the upper tube, changing to dull red or bright crimson before deshiscing. Petals dark purple, elliptic, 1–5 mm long, 0.7–1.5 mm wide in the middle. Filaments 7–15 mm and 5–8 mm long, purple, with pale cream connective; anthers 1.7–2.6 mm long, 1–1.8 mm wide, purple. Style 20–37 mm long, cream to light purple; stigma 1.5–2.1 mm long, 1.1–2 mm wide, yellow-green. Berry oblong to subglobose, 9–11 mm long, 5–7 mm thick, red-purple; seeds oblong-triangular, 0.8–1.1 mm long, 0.4–0.6 mm wide, 240–500 per fruit. *Pistillate flowers*: similar to perfect ones except: floral tube 6–12 mm long, 3.5–5.5 mm wide at rim. Sepals 5–10 mm long, 2.5–3.5 mm wide at base. Petals 1.1–2.4 mm long, 0.3–1.1 mm wide. Filaments 2.5–6 mm and 1.6–5 mm long; anthers aborted, 0.7–1.1 mm long, 0.5–0.8 mm wide. Style 16–30 mm long. Gametic chromosome number $n = 11$ (no meiotic irregularities observed).

Distribution. Widespread from 38°16′S south through the North and South Islands of New Zealand, also Stewart Island, sea level to 300 m; flowering mostly from August to January, occasionally earlier in some localities.

Representative specimens examined. NEW ZEALAND. **Northern North Island:** entrance to Aranui Cave, Waitomo Caverns, *Raven 25193* (CHR, 3 sheets); Mangapu Stream, 5 km NW of Te Kuiti, Waitomo Co., *Gardner 3143* (AK). **Central North Island:** Taumarunui W, Wanganui River Flat, *Atwood in Nov. 1937* (CHR); Ohinewairua, Kaimanawa Mts., *Druce in Apr. 1976* (CHR); Moawhango River, NE of Taihape, *Druce in Oct. 1982* (CHR); Peak Hill, NW Ruahine Range, *Druce in Jan. 1973* (CHR). **Eastern North Island:** Ball's Clearing, Puketitiri, *Druce in Mar. 1972* (CHR); Waipoapoa, S of Havelock North, *Druce in Oct. 1973* (CHR); Maungaharuru Range, SE of Kopua, *Druce in Dec. 1972* (CHR); Dannervirke, *Colenso s.n.* (WELT, 2 sheets). **Southern North Island:** Puketoi Range, S Hawke Bay, *Druce in Dec. 1973* (CHR); Ruakokoputama Valley, Aorangi Range, *Druce in Dec. 1970* (CHR); 2.5 mi. N of Pohangina, *Raven 25129* (CHR, 10 sheets); Merry Hill Farm, Feilding, *Allan in Sep. 1926* (CHR, 6 sheets); Bledisloe Park, Massey, *Raven in 14 Oct. 1969* (CHR); Ohau River, Tararua Range, *Oliver in 6 Apr. 1941* (WELT); Lake Papaitonga, *Mason in 3 Mar. 1942* (CHR, 2 sheets); near Lake Waitawa, N of Otaki, *Duguid in 13 Dec. 1970* (CHR); Silverstream Bush, Hutt Valley, *Druce in Mar. 1976* (CHR); Island Bay, Wellington, *Phillips s.n.* (AK). **Northern South Island:** Kaihoka coast, NW Nelson, *Druce in Nov. 1974* (CHR); 2 mi. E of Te Namu, S of Karamea, *Raven 25238* (CHR); Mokihinui River, *Robins in Dec. 1970* (CHR); Therus Creek Basin, Cobb Valley, NW Nelson, *Druce in Jan. 1979* (CHR); Maitai Valley, Nelson, *Mellor s.n.* (WELT); along D'Urville River near Lake Rotoroa, *Raven 25566* (CHR); Springs Junction, 4 mi. S of Shenandoah, Nelson, *Godley in 3 Feb. 1971* (CHR); Canvastown, Pelorus, *Kirk in 22 Nov. 1886* (WELT); Waiau Valley, *Oliver in 20 Dec. 1947* (CHR, WELT); Haldon Hills, Marlborough, *Given in 31 Dec. 1978* (CHR); Lynton Downs, Kaikoura, *Kirk in Dec. 1889* (WELT). **Eastern South Island:** Gore Bay, N of Hurunui River, *Mason 7861* (CHR); Mt. Cass, SE of Waipara, *Macmillan in 14 Oct. 1962* (CHR); Riccarton Bush, Christchurch, *Raven 35313* (CHR, 36 sheets); Jollie's Bush, Summit Road above Sumner, Banks Peninsula, *Berry & Brako 4624* (CHR); Summit Road above Takamatua Bay, Akaroa Harbour, Banks Peninsula, *Macmillan 68/48* (CHR); Scott's Stream, Mt. Hutt Range, *Cartman in 5 Feb. 1978* (CHR); Alford Forest, S Canterbury, *Simpson 6123* (CHR), *6124* (CHR); Bowyer's Stream, end of Staveley-Sharplin Falls Rd., Alford Forest, *Drury ASH15* (CHR); Winterslow, Taylor's Stream, *Canterbury Bot. Soc. in 8 Jan. 1973* (CHR); Peel Forest, S Canterbury, *Godley in 2 Oct. 1954* (CHR, 3 sheets); ca. 10 mi. E of Geraldine on road to Fairlie, *Raven 25272* (CHR); Hunter's Hills, Blue Cliffs Station, S Canterbury, *Macmillan 72/1355* (CHR), *73/711* (CHR). **Western South Island:** Cape Foulwind, W of Westport, *Berry & Brako 4599* (CHR); 5 mi. N of Punakaiki, *Raven 25227* (CHR); 1 mi. S of Barrytown, *Raven 25220* (CHR, 4 sheets); Lake Ryan Scenic Reserve, Greymouth, *Loh in 13 Nov. 1978* (CHR); Mananui, *Attwood s.n.* (CHR); mouth of Mikonui River, near Ross, *Wardle & Myers in 3 Dec. 1969* (CHR); head of Okarito Lagoon, *Mason & Moar in 24 Feb. 1958* (CHR); S of Lake Mapourika, *Mason & Moar in 17 Feb. 1958* (CHR); N side of Potter's Creek, *Berry & Brako 4606* (CHR); track to Callery Springs, Franz Josef, *Wardle in 2 Dec. 1970* (CHR); 1 mi. N of Paringa River crossing, *Raven 25347* (CHR); 4.2 mi. E of turn to Haast Township, *Raven 25353* (CHR). **Southern South Island:** Port Chalmers, *Cockayne 4109* (WELT); near Dunedin, *Petrie in 1890* (WELT); Otago Peninsula, High Road, ca. 2 mi. E of Centre Road, *Raven 25112* (CHR); Portobello Peninsula, Otago Peninsula, *Johnson in 15 Feb. 1982* (CHR); Waipori Gorge, Otago, *Mason 837* (CHR); Tautuku Bay, Catlins, *Wardle in Nov. 1964* (CHR); Kelso, near Tapanui, Otago, *Petrie in Dec. 1888* (WELT); Spar Bush, Southland, *Powell s.n.* (CHR); Lind's Crossing, Southland, *Kirk in 10 Dec. 1883* (WELT, 3 sheets); Sandy Point, Invercargill, *Raven 25097* (CHR); Bluff, *in Nov. 1907* (WELT 24850). **Stewart Island:** The Neck, near Angela Point, *Wilson 789-559* (CHR).

The lectotype locality of *Fuchsia* ×*colensoi*, Titiokura Saddle, is located in the Moungaharuru Range, ca. 53 km from Napier. Allan (1961: 283) cited *Colenso 144* at K as the type for *F. colensoi*, but his choice is superseded because Hooker only questionably accepted this specimen as belonging to the taxon. Of the five possible syntypes at K, the only one without a clear determination in Hooker's hand is *Colenso 144*, which he annotated in pencil as "*F. Colensoi* ? small var." The other four, including *Colenso 1048*, are annotated by him in ink as *F. colensoi*, without any query.

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Appendix. Index of collections examined.

1. *Fuchsia procumbens* R. Cunningham ex A. Cunningham
2. *Fuchsia cyrtandroides* J. W. Moore
3. *Fuchsia perscandens* Cockayne & Allan
4. *Fuchsia excorticata* (J. R. & G. Forster) Linnaeus f.
5. *Fuchsia ×colensoi* Hooker f.

Adams s.n. (AK 15112, 15113) **4**, s.n. (AK) **1**, s.n. (WELT) **1**; Alford in 26 Apr. 1934 (WELT) **4**; Allan 173 (CHR) **3**, 394 (CHR) **3**, 982a (CHR) **3**, in Sep. 1926 (CHR 11519–11524) **5**, s.n. (CHR 11526) **4**, s.n. (WELT 8379, mixed collection with **4** and **5**) **3**, 29365 (CHR) **1**; Anderson in 2 Jan. 1971 (CHR) **4**; Aston in Jan. 1909 (AK, WELT) **4**, s.n. (AK 5899) **4**, s.n. (WELT) **4**; Atkinson in 20 Feb. 1931 (WELT) **3**, in 12 Jan. 1964 (CHR) **4**; Atwood in Nov. 1937 (CHR) **5**, in Jan. 1940 (AK) **3**, s.n. (AK) **5**.

Banks & Solander s.n. (AK 101152) **4**; Bartlett N40 (CHR) **1**, TV181 (CHR) **3**, s.n. (CHR 314556) **4**; Bellingham s.n. (AK 168940) **4**; Berry & Brako 4596 (CHR) **4**, 4598 (CHR) **3**, 4599 (CHR) **5**, 4600 (CHR) **5**, 4601 (CHR) **5**, 4602 (CHR) **4**, 4603 (CHR) **5**, 4605 (CHR) **5**, 4606 (CHR) **5**, 4608 (CHR) **5**, 4609 (CHR) **3**, 4611 (CHR) **3**, 4612 (CHR) **5**, 4613 (CHR) **5**, 4614 (CHR) **5**, 4615 (CHR) **4**, 4622 (CHR) **3**, 4624 (CHR) **5**, 4626 (AK, MO) **1**, 4627 (AK, MO) **1**; Berry et al. 4587 (CHR) **3**, 4588 (CHR) **5**, 4589 (CHR) **5**, 4590 (CHR) **5**, 4591 (CHR) **5**, 4628 (PAP, MO) **2**; Bluff in Nov. 1907 (WELT 24850) **5**; Bowman in 26 Dec. 1975 (CHR) **4**, s.n. (AK 151199) **4**; Brownlie 84 (CHR) **4**; Burke, G.K.R. & W.D., s.n. (WELTU) **1**; Burrell s.n. (CHR 133367) **4**.

Canterbury Bot. Soc. in 13 Mar. 1971 (CHR) **5**, in 8 Jan. 1973 (CHR) **5**; Carlquist 653 (DS—2 sheets, RSA) **2**; Carse 980/4 (CHR) **4**, 980/8 (CHR) **4**, 980/11 (CHR) **4**, 982 (CHR) **1**, 982/A (CHR) **1**, s.n. (CHR) **1**, s.n. (WELT) **1**; Cartman in 5 Feb. 1978 (CHR) **5**; Chapman in 2 Oct. 1973 (CHR) **4**, s.n. (CHR 239330) **4**; Cheeseman in Jan. 1875 (AK, 2 sheets) **3**, in Jan. 1884 (AK) **3**, s.n. (AK 101154) **1**, s.n. (AK 101155) **1**, s.n. (AK 5895) **4**, s.n. (AK) **1**, s.n. (AK, 2 sheets; WELT) **1**; Cockayne 4109 (WELT) **5**; Colenso 1048 (K) **5**, s.n. (WELT 22767, mixed collection with **5**) **3**, s.n. (WELT, 2 sheets) **5**; Collett in Mar.–Apr. 1965 (CHR) **3**; Collis in 14 Nov. 1964 (AK) **3**; Cooper s.n. (AK 117989) **4**, s.n. (AK 117990) **4**, s.n. (AK 118459) **4**, s.n. (AK 118991) **4**, s.n. (AK 119899) **4**, s.n. (AK 120089) **4**, s.n. (AK 120225) **4**, s.n. (AK 125670) **4**, s.n. (AK 125671) **4**, s.n. (AK 125711) **4**, s.n. (AK 125760) **4**, s.n. (AK 126195) **4**, s.n. (AK 126196) **4**, s.n. (AK 126354) **4**, s.n. (AK 126467) **4**, s.n. (AK 126801) **4**, s.n. (AK 127333) **4**, s.n. (AK 127370) **4**, s.n. (AK 127371) **4**, s.n. (AK 128220 and AK 128223) **4**, s.n. (CHR 194295) **4**, s.n. (CHR 194296) **4**; Court s.n. (AK 149877) **4**; Cragg in 22–28 Mar. 1973 (CHR) **4**; Cunningham, R. (K) **1**.

Davey s.n. (CHR 194272) **4**; Davidson in 1909 (WELT) **4**; Davidson & Petrie in Nov. 1908 (WELT) **3**; Dawbin in 25 Aug. 1943 (CHR) **4**; de Lange in 21 Oct. 1984 (AK) **3**, in 6 Feb. 1985 (AK) **3**, in 4 Dec. 1991 (AK) **3**; Dickson s.n. (AK 140298) **4**, s.n. (AK, CHR) **1**; Dickinson & Mark in 24 Jan. 1986 (CHR, OTA) **3**; Druce in Jan. 1966 (CHR) **3**, in Feb. 1967 (CHR) **3**, in Dec. 1967 (CHR) **3**, in Feb. 1970 (CHR) **4**, in Nov. 1970 (CHR) **4**, in Dec. 1970 (CHR) **5**, in Mar. 1971 (CHR) **3**, in June 1971 (CHR) **3**, in Feb. 1972 (CHR) **5**, in Mar. 1972 (CHR) **5**, in Oct. 1972 (CHR) **3**, in Nov. 1972 (CHR) **3**, in Nov. 1972 (CHR) **5**, in Dec. 1972 (CHR) **5**, in Jan. 1973 (CHR) **3**, in Jan. 1973 (CHR) **5**, in Apr. 1973 (CHR) **4**, in June 1973 (CHR) **3**, in Oct. 1973 (CHR) **3**, in Oct. 1973 (CHR) **4**, in Oct. 1973 (CHR) **5**, in Dec. 1973 (CHR) **5**, in Jan. 1974 (CHR) **4**, in Nov. 1974 (CHR) **3**, in Nov. 1974 (CHR) **4**, in Nov. 1974 (CHR) **5**, in Nov. 1975 (CHR) **3**, in Feb. 1976 (CHR) **5**, in Mar. 1976 (CHR) **3**, in Mar. 1976 (CHR) **5**, in Apr. 1976 (CHR) **5**, in Oct. 1976 (CHR) **3**, in Nov. 1976 (CHR) **3**, in Jan. 1977 (CHR) **4**, in Apr. 1977 (CHR) **3**, in Oct. 1978 (CHR) **3**, in Dec. 1978 (CHR) **3**, in Jan. 1979 (CHR) **5**, in Feb. 1979 (CHR) **3**, in Nov. 1979 (CHR) **3**, in Apr. 1980 (CHR) **3**, in June 1982 (CHR) **3**, in Oct. 1982 (CHR) **3**, in Oct. 1982 (CHR) **5**, s.n. (CHR 129787) **4**, s.n. (CHR 129788) **4**, s.n. (CHR 158729) **4**, s.n. (CHR 180891) **4**, s.n. (CHR 208680) **4**, s.n. (CHR 210044) **4**, s.n. (CHR 210450) **4**, s.n. (CHR 244274) **4**, s.n. (CHR 244927) **4**, s.n. (CHR 262318) **4**, s.n. (CHR 282807) **4**, s.n. (CHR 86602) **4**, s.n. (CHR) **3**; Drury ASH15 (CHR) **5**; Duguid in 18 Oct. 1953 (CHR) **3**, in 13 Dec. 1953 (CHR) **3**, in 13 Dec. 1970 (CHR) **5**, s.n. (CHR 194263) **4**, s.n. (CHR 194268) **4**, s.n. (CHR 194269) **4**, s.n. (CHR 194270) **4**, s.n. (CHR 194271) **4**, s.n. (CHR 194274) **4**, s.n. (CHR 194275) **4**.

Elder 723/2 (CHR) **3**; Engelhorn 15282 (CHR) **5**, 25284 (CHR) **3**, 25714 (CHR) **1**, 25719 (CHR, 3 sheets), 25720 (CHR) **1**, in 25 Nov. 1969 (CHR) **4**; Enting in 13 Apr. 1973 (WELT) **4**; Ericson in 12 Dec. 1970 (CHR) **3**, in 9 Dec. 1971 (CHR) **3**; Esler 3629 (CHR) **1**; Esler 3630 (CHR) **1**, in Jan. 1978 (OTA) **4**; Evetts (AK 135484) **4**.

Florence 4700 (BISH, K, NY, P, PAP, US) **2**, 5455

(BISH, P, PAP, US) **2**, 7631 (BISH, K, P, PAP) **2**, 7748 (PAP) **2**; Florence & Guérin 2713 (PAP—2 sheets) **2;** Flynn & Flynn 329 (PAP) **2**; Forester s.n. (AK 168954) **4**; Forster s.n. (LINN, Herb. Smith 670.7) **4**; Fosberg 61095 (CHR) **2**, 62646 (CHR) **2**; Frater et al. 355 (AK) **4**, s.n. (AK) **4**.

Gardner 3142 (AK, WELT) **3**, 3143 (AK) **5**, H164 (AK) **3**; Given 69543 (CHR) **4**, 69879 (CHR) **4**, 70232 (CHR) **4**, 70590 (CHR) **4**, 70598 (CHR) **4**, 70685 (CHR) **4**, 70696 (CHR) **4**, 70771 (CHR) **4**, 72207 (CHR) **4**, 72381 (CHR) **4**, 72545 (CHR) **4**, 72600 (CHR) **4**, 72652 (CHR) **4**, in 31 Dec. 1978 (CHR) **5**; Given & Bell 70652 (CHR) **4**; Given & Williams 13023 (CHR) **4**; Godley G1785 (CHR, 2 sheets) **1**, G1786 (CHR, 2 sheets) **1**, G1787 (CHR, 2 sheets) **1**, G2201 (CHR) **1**, G2202 (CHR, 2 sheets) **1**, G2203 (CHR, 2 sheets) **1**, G2206 (CHR, 2 sheets) **1**, G2207 (CHR) **1**, G2208 (CHR, 2 sheets) **1**, G2209 (CHR, 2 sheets) **1**, G2210 (CHR) **1**, G2211 (CHR) **1**, in 2 Oct. 1954 (CHR, 3 sheets) **5**, in 29 Dec. 1962 (CHR) **4**, in 3 Feb. 1971 (CHR) **4**, in 3 Feb. 1971 (CHR) **5**, s.n. (CHR 117496) **1**; Godley & Smith 56 (CHR) **3**; Goulding 761 (AK) **4**, 762 (AK) **4**; Grace & Scott s.n. (AK 162897) **4**; Grant 3742 (A, BISH, K) **2**.

Hag s.n. (CHR 73525) **4**; Hamel in 3 Nov. 1970 (CHR) **4**, in 28 Jan. 1976 (CHR, OTA) **4**, in 29 Jan. 1976 (OTA) **4**; Hamilton s.n. (CHR 194294) **4**, s.n. (CHR) **4**; Hamlin 919 (WELT) **4**, s.n. (CHR 81823) **4**; Healy 1023 (CHR) **3**, 173 (CHR) **3**, 443 (CHR) **3**, 444 (CHR) **3**, in 31 Dec. 1932 (WELT) **4**, in 3 Oct. 1934 (CHR, WELT) **3**, in 1 Nov. 1940 (CHR) **3**, in 20 Dec. 1943 (CHR) **3**, in 27 Feb. 1944 (CHR) **3**, s.n. (CHR) **3**, s.n. (WELT) **3**, s.n. (WELT) **4**; Hector in Dec. 1862 (K) **5**; Hedditch in Nov. 1966 (CHR) **3**; Heginbotham in Aug. 1975 (CHR) **4**; Heine 14 Jan. 1930 (WELT) **4**, in 13 Nov. 1931 (WELT, 2 sheets) **4**, in 29 Dec. 1932 (WELT) **4**; Holloway in 19 Nov. 1980 (CHR) **3**, s.n. (OTA) **3**; Hutson s.n. (CHR 67808) **4**; Hynes in 23 Dec. 1966 (AK) **3**, in 28 Jan. 1967 (AK) **5**, in 16 Feb. 1969 (CHR) **4**, s.n. (AK 104136) **4**, s.n. (AK 110353) **4**, s.n. (AK 117275) **4**, s.n. (AK 120408) **4**, s.n. (AK 129117) **4**, s.n. (AK 144190) **4**; Hynes & Lynch s.n. (AK 126715) **4**.

Johnson in 17 Oct. 1971 (OTA) **4**, in 20 Oct. 1981 (CHR) **3**, in 15 Feb. 1982 (CHR) **5**, s.n. (CHR 261595) **4**.

Kelly in 23 Sep. 1965 (CHR) **3**, in 28 Oct. 1965 (CHR) **3**, in 18 Nov. 1965 (CHR) **4**; Kelly & Kelly in 10 Oct. 1973 (CHR) **3**; Kennedy s.n. (WELT) **4**; Kirk 62 (K) **1**, 79 (WELT) **1**, 192 (AK, CHR) **4**, 194 (CHR) **1**, in 10 Dec. 1883 (WELT, 3 sheets) **5**, in 8 Nov. 1883 (WELT, 3 sheets) **4**, in 22 Nov. 1886 (WELT) **3**, in 22 Nov. 1886 (WELT) **4**, in 22 Nov. 1886 (WELT) **5**, in Dec. 1889 (WELT) **4**, in Dec. 1889 (WELT) **5**, s.n. (AK 11473) **1**, s.n. (AK 15117) **1**, s.n. (AK 5909) **1**, s.n. (CHR) **1**, s.n. (WELT 24809 and 24837) **1**, s.n. (WELT 24823) **1**, s.n. (WELT) **1**, s.n. (WELT) **3**.

Lambrechtsen in 11 Jan. 1971 (CHR) **4**, in 12 Jan. 1971 (CHR) **4**, in 14 Jan. 1971 (CHR) **4**, s.n. (CHR 367024) **4**, s.n. (CHR 367025) **4**, s.n. (CHR 367026) **4**, s.n. (CHR 367027) **4**; Langbern 1/2 (CHR) **3**; Leahy s.n. (AK 137014) **4**; Lindsay in 6 Nov. 1861 (K) **5**; Lloyd s.n. (CHR) **1**; Lloyd, R. 71615 (CHR) **1**; Loh in 13 Nov. 1978 (CHR) **5**, in 16 Nov. 1978 (CHR) **4**, in 21 Nov. 1978 (CHR) **3**; Lush in 25 Mar. 1951 (CHR) **3**.

MacDaniels 1315 (BH, BISH) **2**, 1341 (BISH) **2**; Maclet 156 (CHR) **2**; Macmillan 68/35 (CHR) **3**, 68/47 (CHR) **3**, 68/48 (CHR) **5**, 69/56 (CHR) **3**, 70/269 (CHR) **3**, 70/277 (CHR) **4**, 70/288 (CHR 215233) **4**, 72/1021 (CHR) **4**, 72/1226 (CHR) **3**, 72/1355 (CHR) **5**, 72/919 (CHR) **3**, 73/711 (CHR) **5**, 79/193 (CHR) **3**, in 14 Oct. 1962 (CHR) **5**, in 12 Dec. 1962 (CHR) **4**, in July 1964 (CHR) **4**, in 30 Sep. 1970 (CHR) **4**, in 25 Jan. 1972 (CHR) **4**; Macmillan & Stemmer in 29 Jan. 1973 (CHR) **4**; Macmillan & Woodhouse 72/1200 (CHR) **3**; Manning in 15 Dec. 1984 (CHR) **4**; Mark & Wells in 1 Dec. 1966 (OTA) **4**; Mason 11831 (CHR) **4**, 11860 (CHR) **4**, 12986 (CHR) **5**, 7861 (CHR) **5**, 837 (CHR) **5**, 8876A (CHR 121153) **4**, in 10 Jan.1944 (CHR) **5**, in 11 Nov. 1945 (CHR, 2 sheets) **3**, in 3 Mar. 1942 (CHR, 2 sheets) **5**, in 31 Mar. 1945 (CHR) **5**, in 1 Dec. 1945 (CHR) **3**, in 1 Dec. 1945 (CHR) **5**; Mason & Moar 1713 (CHR) **3**, 1714 (CHR) **3**, 5714 (CHR) **3**, in 17 Feb. 1958 (CHR) **5**, in 24 Feb. 1958 (CHR) **5**; Mason & Simpson in 22 Nov. 1960 (CHR) **4**; Matthews 15 (AK) **4**, 2310 (AK) **4**, 2311 (AK) **4**, s.n. (AK 5898) **4**, s.n. (AK) **1**; Matthews, H.B. & Carse 982/2 (CHR) **1**, 982/4 (CHR) **1**; Matthews, R.H., 982/1 (CHR) **1**, 982/3 (CHR) **1**, s.n. (AK) **1**; Mellor s.n. (WELT) **5**; Meurk in 24 Sep. 1971 (CHR) **3**; Miller in 17 Apr. 1976 (OTA) **4**; Molloy in 17 Nov. 1976 (CHR) **5**, in 4 Mar. 1988 (CHR) **4**; Moore 22139 (CHR) **1**, in 11 Oct. 1969 (CHR) **4**, in 12 Oct. 1953 (CHR, 2 sheets) **3**, in 26 Dec. 1957 (CHR) **3**, in 3 Jan. 1971 (CHR) **4**.

Ogle 57 (CHR) **3**, 96 (CHR) **1**, 386 (CHR) **1**, 387 (CHR) **1**, 430 (WELT) **3**, 434 (WELT) **3**, 563 (CHR) **3**, 726 (CHR) **3**, 1381 (CHR) **3**, in 12 Sep. 1974 (WELT) **4**; Oliver in 16 Nov. 1926 (WELT) **4**, in 27 Mar. 1927 (WELT) **4**, in 10 Oct. 1930 (WELT) **3**, in 7 Dec. 1930 (WELT, 3 sheets) **4**, in Dec. 1940 (WELT) **4**, in 6 Apr. 1941 (WELT) **5**, in 12 Oct 1945 (WELT) **3**, in 13 Dec. 1947 (CHR, WELT) **3**, in 20 Dec. 1947 (CHR, WELT) **5**, in 23 Nov. 1947 (CHR, WELT) **3**, in 10 Apr. 1948 (CHR, WELT) **3**, in 10 Apr. 1948 (CHR) **5**, in 16 Apr. 1948 (WELT) **3**, in 17 Apr. 1948 (CHR, WELT) **3**, in 29 Aug. 1948 (WELT) **3**, in 6 Sep 1948 (WELT) **3**, in 1 Oct. 1949 (WELT) **3**, in 11 Feb. 1953 (WELT) **4**; Olsen 2 (AK) **1**, N16/90–31 (AK) **1**; Olsen s.n. (AK) **1**, s.n. (Godley G2204, CHR, 2 sheets) **1**; Orchard 3553 (AK) **4**, 3613 (AK) **4**, 3772 (AK) **4**, 4037 (AK) **4**; Osborne s.n. (AK, 2 sheets, male) **1**.

Parris & Keen s.n. (AK 110746) **4**; Parsons 225 (CHR) **4**, 226 (CHR) **4**; Petrie in Dec. 1886 (WELT, mixed collection with **5**) **3**, in Dec. 1888 (WELT) **5**, in 1890 (WELT) **5**, in Oct. 1892 (WELT) **3**, in Nov. 1894 (WELT) **4**, in Sep. 1896 (WELT) **4**, in 29 Oct. 1898 (WELT) **4**, in Nov. 1906 (WELT) **4**, in Nov. 1908 (WELT) **3**, s.n. (WELT) **4**; Phillips s.n. (AK) **3**, s.n. (AK) **5**; Poole in 8 Jan. 80 (CHR) **3**; Powell s.n. (AK 40243, 44878) **4**, s.n. (CHR) **5**.

Rattenbury s.n. (AK 169927) **4**; Raven 25097 (CHR) **5**, 25112 (CHR) **5**, 25118 (CHR) **4**, 25121 (CHR, 7 sheets) **3**, 25122 (CHR) **4**, 25123 (CHR, 3 sheets) **3**, 25126–1 (CHR) **3**, 25127 (CHR, 10 sheets) **3**, 25128 (CHR) **4**, 25128–2 (CHR) **4**, 25128–3 (CHR) **4**, 25129 (CHR, 10 sheets) **5**, 25130 (CHR) **4**, 25132 (CHR) **3**, 25134 (CHR, 2 sheets) **4**, 25135 (CHR) **4**, 25136 (CHR) **5**, 25137 (CHR) **5**, 25145 (CHR) **1**, 25147 (CHR) **1**, 25181 (CHR) **4**, 25187 (CHR) **4**, 25192–2 (CHR) **3**, 25192–4 (CHR) **3**, 25193 (CHR, 3 sheets) **5**, 25203 (CHR) **4**, 25206–1 (CHR) **3**, 25212 (CHR, 8 sheets) **3**, 25215 (CHR, 4 sheets) **3**, 25216 (CHR, 4 sheets) **5**, 25219 (CHR) **4**, 25220 (CHR, 4 sheets) **5**, 25222 (CHR) **4**, 25223 (CHR) **5**, 25224 (CHR, 7 sheets) **3**, 25227 (CHR) **5**, 25232 (CHR) **5**, 25238 (CHR) **5**, 25239 (CHR, 10 sheets) **3**, 25271 (CHR) **4**, 25272 (CHR) **5**, 25334 (CHR) **4**, 25337 (CHR) **4**, 25346 (CHR) **3**, 25347 (CHR) **5**, 25353 (CHR) **5**, 25358 (CHR) **4**, 25414 (CHR) **4**,

25420 (CHR) **4**, 25431 (CHR) **4**, 25566 (CHR) **5**, 25567 (CHR, 2 sheets) **3**, 35313 (CHR, 36 sheets) **5**, et al. 25214 (CHR) **4**, in 14 Oct. 1969 (CHR) **5**; Rawlings s.n. (CHR) **1**; Rawson in 2 Oct. 1947 (CHR) **4**, in 20 Jan. 1947 (CHR) **4**, in 1954 (CHR, 4 sheets) **3**; Reynolds 155441 (CHR) **1**; Ritchie in 22 Feb. 1966 (CHR) **4**; Ritchie & Ritchie in 16 Sep. 1968 (CHR) **4**; Robins in Dec. 1970 (CHR) **5**; Robins & Mitchell in 6 Jan. 1969 (CHR) **3**.

Sachet 2352 (MO, US) **2**; Salmon in Dec. 1943 (WELT) **4**; Scofield 13 (AK) **4**; Shakespear s.n. (AK 127539) **4**; Simpson 1365 (CHR) **4**, 5505 (CHR) **4**, 6123 (CHR) **5**, 6124 (CHR) **5**, 6628 (CHR) **5**, 7076 (CHR) **5**, 7459 (CHR) **3**, 8251 (CHR) **3**, in 20 Jan. 1968 (CHR) **5**; Simpson & Chapman 6926 (CHR) **3**; Simpson & Macmillan in 20 Jan 1968 (CHR) **3**; Smith 165 (CHR) **3**, 166 (CHR) **5**, 188 (CHR) **2**, in 8 Feb. 1974 (CHR) **5**, s.n. (AK 118077, 109157) **4**; Smith-Dodsworth s.n. (AK) **1**; St. John & Fosberg 17005 (BISH) **2**; Sykes in 14 Apr. 1971 (CHR) **4**.

Talbot in 1947 (CHR) **3**, in Jan. 1949 (CHR) **3**, s.n. (CHR) **3**; Taylor in 29 Nov. 1970 (CHR) **4**; Thomson 981/1 (CHR) **5**; Travers s.n. (K) **3**; Trevarthen s.n. (AK) **1**; Troup s.n. (AK 119584) **4**; Tryon s.n. (AK, mixed with **3**) **5**.

Veillon & Guérin 38 (BISH, P, PAP, US) **2**; Veitch s.n. (CHR 191554) **4**.

Wanganui Museum Botanical Group 40 (CHR) **4**; Wardle in Nov. 1964 (CHR) **5**, in 2 Nov. 1966 (CHR) **4**, in 30 Nov. 1970 (CHR) **4**, in 2 Dec. 1970 (CHR) **5**, in 4 Dec. 1970 (CHR) **3**, in 5 Dec. 1970 (CHR) **3**, in 5 Jan. 1975 (CHR) **3**, in 8 Dec. 1975 (CHR) **3**, in 7 Jan. 1977 (CHR) **4**, in 31 Mar. 1977 (CHR) **3**, in 10 Jan. 1985 (CHR) **5**; Wardle & Fryer in 4 Nov. 1968 (CHR) **3**; Wardle & Myers in 3 Dec. 1969 (CHR) **3**, in 3 Dec. 1969 (CHR) **5**; Wilson 789–559 (CHR) **5**, 789–85 (CHR) **4**; Wood in 7 Jan. 1954 (AK 32524, mixed collection with **5**) **3**, in 29 Nov. 1987 (CHR) **4**, s.n. (AK 27666) **4**, s.n. (AK 118587) **4**; Wright 2226 (AK) **4**, 3505 (AK) **4**, 3687 (AK) **4**, 512 (AK) **4**, 5332 (AK) **1**, 5389 (AK) **1**, 5510 (AK) **1**, 5600 (AK) **1**, 633 (AK) **4**, 7094 (AK) **1**, 781 (AK) **4**.

Yaldwyn in 1 Mar. 1972 (WELT) **4**.

Zotov 193 (CHR) **4**, in 1 Oct. 1929 (CHR) **3**, in 6 Oct. 1929 (CHR) **5**, in 3 Mar. 1962 (CHR, 2 sheets) **4**, in 28 Dec. 1972 (CHR) **3**.

POLLINATION BIOLOGY OF *LAPEIROUSIA* SUBGENUS *LAPEIROUSIA* (IRIDACEAE) IN SOUTHERN AFRICA; FLORAL DIVERGENCE AND ADAPTATION FOR LONG-TONGUED FLY POLLINATION[1]

Peter Goldblatt,[2] John C. Manning,[3] and Peter Bernhardt[4]

Abstract

Lapeirousia subg. *Lapeirousia* (Iridaceae) consists of 21 species endemic to sub-Saharan Africa, with the Mediterranean southwestern part of southern Africa the center of diversity. Analysis of the floral ecology of 19 species shows that the majority secrete large amounts of sucrose-rich or sucrose-dominant nectar, and the 16 species for which pollinator observations were obtained are cross-pollinated exclusively by insects with elongated mouth parts, representing three orders (Diptera, Hymenoptera, and Lepidoptera). In the absence of pollinators, at least four species (*L. arenicola, L. jacquinii, L. oreogena, L. simulans*) were found to set seed without cross pollination and are assumed to be mechanically autogamous. The 20 species segregate into three pollination types based on divergent patterns of pigmentation, scent production, perianth tube length, and pollinator taxa. Species with flowers of the *L. silenoides*-type are pollinated exclusively by *Prosoeca* species (Diptera: Nemestrinidae), and species with flowers of the *L. fabricii*-type are pollinated exclusively by *Moegistorhynchus longirostris* (Diptera: Nemestrinidae) and *Philoliche gulosa* (Diptera: Tabanidae). The latter two fly species, together with the *Prosoeca* species, have the longest mouth parts of all pollinators examined on species of subgenus *Lapeirousia*. The *L. divaricata*-type flower is pollinated by a combination of bees, mostly female Anthophoridae (e.g., *Anthophora, Amegilla, Tetraloniella*) and native *Apis mellifera* (Apidae), and to a lesser extent by bombyliid flies and some Lepidoptera. Observations of insect pollen load analysis suggest that the evolution of these three flower types has encouraged ethological isolation between species, but is a small component in broader pollination guilds encompassing co-blooming species in other genera and families, including Asteraceae, Geraniaceae, Scrophulariaceae, and Sterculiaceae. Mapping of pollination syndromes on a phylogenetic tree of subgenus *Lapeirousia* demonstrates the extreme adaptive radiation in the subgenus and the convergent development of the same pollination strategy repeatedly across the subgenus.

Africa is the center of diversity for the family Iridaceae, with the majority of species concentrated in the temperate and Mediterranean regions in the southern part of the continent (Goldblatt, 1978, 1994). One striking feature of the iridaceous flora of southern Africa is the variation in floral form and color (see Marloth, 1917–1932; Jeppe, 1989). By far the greater proportion of species of Iridaceae in southern Africa have flowers in which the perianth forms an elongated tube and the stamens and style are often prominently displayed. Although the tepals of such flowers do not have the characteristic shape found in *Iris* or *Moraea*, these tubular flowers exhibit a wide variety of colors, color patterns, and tepal orientation. The floral ecology of Iridaceae with exaggerated floral tubes has received less attention than those genera of the family with gullet blossoms (sensu Faegri & van der Pijl, 1979).

Within the southern African Iridaceae there are genera with more typical *Iris*-type flowers in which each flower is a meranthium. That is, each flower functions as three separate reproductive units, each

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consisting of a stamen and a stigma hidden between ornate style crests and a spreading tepal (e.g., *Moraea, Dietes, Gynandriris*). Initial work on the floral ecology of *Moraea* (Vogel, 1954; Goldblatt et al., 1989) suggested that this genus has a pollination mechanism similar to those *Iris* species in the Northern Hemisphere (Faegri & van der Pijl, 1979) in which the pollination biology has been studied. Here heavy insects (usually bees and wasps) depress the drooping tepal to probe the floral interior for nectar and contact the sexual organs of the flower.

Most of the literature on the pollination ecology of tubular-flowered Iridaceae in southern Africa consists of little more than brief descriptions (Scott Elliot, 1890, 1891; Marloth, 1908, 1917–1932; Vogel, 1954; Johnson, 1992). This literature suggests that Iridaceae with tubular flowers may be pollinated by a wider variety of animals with much elongated mouth parts, including sunbirds, bees, flies in the families Nemestrinidae, Tabanidae, and Bombyliidae, sphinx moths, and other Lepidoptera, notably *Meneris tulbaghia* (Satyridae). Both the taxonomic literature and the brief reports of pollination observations indicate that tubular flowers specialized for pollination by these vectors have evolved in many families distributed through southern Africa, including the Amaryllidaceae, Crassulaceae, Ericaceae, Geraniaceae, Orchidaceae, Proteaceae, Scrophulariaceae, etc. (Vogel, 1954; Johnson, 1992; Johnson & Bond, 1994; Johnson et al., 1993).

Long-term fieldwork and quantitative analysis of the floral ecology of tubular-flowered Iridaceae has been confined so far to work on the genus *Nivenia* (Goldblatt & Bernhardt, 1990). Flowers in this genus are homostylous or heterostylous and are pollinated primarily by long-tongued flies in the genus *Prosoeca* (Nemestrinidae) and secondarily by female anthophorid bees. Is this pollination syndrome characteristic of other tubular-flowered Iridaceae in southern Africa?

Lapeirousia represents a far more promising genus for fieldwork on floral ecology of the African Iridaceae. This genus consists of 41 species in two subgenera, of which subgenus *Lapeirousia* has the more visibly diverse and specialized floral forms. Subgenus *Lapeirousia* is also easier to study, since many of its species have a narrowly circumscribed distribution, compared with the almost pan-sub-Saharan African subgenus *Paniculata* (Goldblatt, 1990). Nineteen of the 21 species in subgenus *Lapeirousia* occur along the west coast and near interior of southern Africa, and flowering sites for most species are easily accessible (Goldblatt, 1972; Goldblatt & Manning, 1994). The other two species, *L. littoralis* Baker and *L. odoratissima* Baker, are widespread across south tropical Africa, and are thus outside our study area. To elucidate the dynamics of pollination in *Lapeirousia*, observations will be compared with results of the earlier study of *Nivenia* and with what is known about the pollination ecology of tubular flowers of southern Africa in general.

Materials and Methods

Lapeirousia species grow in a variety of habitats ranging from montane areas to coastal flats, and in various soils, including coarse sands of various origins, shales and clays, and granites. Plants are often fairly common locally, but species have distribution patterns ranging from extremely local to widespread. Species of subgenus *Lapeirousia* include local endemics such as *L. verecunda* and *L. simulans*, both known from single extended populations in Namaqualand, and widespread species such as *L. anceps*, *L. fabricii*, and *L. pyramidalis*, that extend over half the entire southern African range of the subgenus. The species studied, study sites, and voucher information are listed in Table 1. Voucher specimens were made for all populations of *Lapeirousia* studied, also for species of other plant taxa observed to be visited by the same insect taxa collected on *Lapeirousia*. Plant vouchers are deposited at the Missouri Botanical Garden Herbarium, St. Louis (MO), and the Compton Herbarium, Cape Town (NBG).

Observation of insect foraging involved 4–20 hours per plant species from 1992 to 1994, and included aspects such as the frequency (number of visits per unit time) and taxonomic diversity of floral foragers, and how they removed rewards from flowers. When observed to probe the floral tube or contact the anthers or stigma, insects were captured and killed in a jar using ethyl acetate fumes. Location of pollen deposits was based on the examination of pinned insects. Pollen was removed from individual insects after pinning by placing the insect on a glass slide and gently rinsing the whole body in 100% ethanol while gently dislodging pollen loads on the frons, thorax, and in the case of bees, hind legs, with a dissecting needle. When the ethanol had evaporated the pollen residue was stained and mounted in 1–2 drops of Calberla's fluid (Ogden et al., 1974). To prevent contamination of the body of an insect with pollen carried by another in the same killing jar, the bodies of insect specimens were isolated from each other by wrapping them in tissue. The pollen of a plant species was scored as present on the body of an insect if

Table 1. Species of subgenus *Lapeirousia* studied, with voucher and study site information. All sites are in the Western Cape and Northern Cape Provinces, South Africa; voucher specimens are located at MO and NBG. Asterisks (*) mark main study site and source of nectar sugar data in Tables 3 and 4 where there is more than one study site.

Species	Study site and voucher information
L. anceps (L.f.) Ker-Gawl.	Near Ysterfontein, N of Cape Town, *Manning s.n.* (no voucher)*; Olifants River Valley, 15 km N of Clanwilliam, *Goldblatt & Manning 9697* (no voucher)
L. arenicola Schltr.	Knersvlakte, 10 km N of Vanrhynsdorp, *Goldblatt & Manning 9453*
L. exilis Goldblatt	Ratelpoort, N of Springbok, *Goldblatt & Manning 9897*
L. barklyi Bak.	Richtersveld, dunes W of Grasvlakte, *Goldblatt & Manning 9460*
L. dolomitica Dinter subsp. *dolomitica*	Richtersveld, hills above farm Grasvlakte, *Goldblatt & Manning 9308*
subsp. *lewisiana* B. Nord.	Namaqualand, 10 km S of Garies, *Goldblatt & Manning 9188*
L. divaricata N. E. Br.	Bidouw Valley, alluvium at foot of pass, *Goldblatt 9190**; valley bottom between Doornbosch and Bidouw, *Goldblatt & Manning 9633*
L. fabricii (Delaroche) Ker-Gawl.	Top of Spektakel Pass, W of Springbok, *Goldblatt & Manning 9711**; Olifants River Valley, near Citrusdal, *Goldblatt & Manning 10030*
L. jacquinii N. E. Br.	Farm Driefontein, S of Nieuwoudtville, *Goldblatt & Manning 9412**; Top of Botterkloof Pass, *Goldblatt & Manning 9966*
L. macrospatha Bak.	Richtersveld, farm Grasvlakte, sandy plain, *Goldblatt & Manning 9455*
L. montana Klatt	Klein Roggeveld, foot of Komsberg Pass, *Goldblatt & Manning 9672C*
L. oreogena Schltr. ex Goldblatt	Glenlyon farm, Nieuwoudtville, *Goldblatt & Manning 9643A*
L. plicata (Jacq.) Diels	Flats below the Hantamsberg, Calvinia, *Goldblatt & Manning 9912*
L. pyramidalis (Lam.) Goldblatt subsp. *pyramidalis*	Hill W of Worcester, *Goldblatt & Manning 9592**; mountain slopes E of De Wet, *Goldblatt & Manning s.n.* (no voucher); foot of Hex River Pass, *Goldblatt & Manning 9435A*
subsp. *regalis* Goldblatt & Manning	Olifants River Valley opposite Trawal, *Goldblatt & Manning 9909**; Olifants River Valley near Klawervlei, *Goldblatt & Manning 9223*
L. silenoides (Jacq.). Ker-Gawl.	Namaqualand, between Garies and Kamieskroon, *Goldblatt & Manning 9467**; Spektakel Pass, W of Springbok, *Goldblatt & Manning 9327*
L. simulans Goldblatt & Manning	Knersvlakte, 10 km N of Vanrhynsdorp, *Goldblatt & Manning 9454*
L. spinosa (Goldblatt) Goldblatt & Manning	Richtersveld, near farm Grasvlakte, *Goldblatt & Manning 9456*
L. tenuis (Goldblatt) Goldblatt & Manning	Plains 62 km W of Springbok to Grootmis, *Goldblatt & Manning 9935*
L. verecunda Goldblatt	E slopes of Spektakel Pass, *Goldblatt & Manning 9144*
L. violacea Goldblatt	Top of Botterkloof Pass, *Middlemost 1605*

more than 10 individual grains (or polyads) were counted on the slide (Table 2).

Field estimates of nectar volume were made from unbagged flowers, thus nectar volumes (Table 4) represent the result of secretion and exploitation. To extract nectar, flowers were picked and nectar was withdrawn from the base of the floral tube with 3-μl capillary tubes after separating the ovary from the perianth. Nectar samples were dried on Whatmans filter paper no. 1 and sent to B.-E. van Wyk, Rand Afrikaans University, Johannesburg, for analysis (Table 3). The percentage of sugars dissolved in fresh nectar (Table 4) was recorded on a Bellingham & Stanley hand-held refractometer (0–50%) using nectar extracted from flowers in the manner described above. Most nectar sugars were analyzed in the field, but a few samples were taken from flowers of cut stems placed in water. Flowers of *Lapeirousia* last well in water, and the chemistry of nectar sugars was evidently not affected by the latter treatment, but nectar volumes were found to be higher than in specimens examined in the field, presumably because visitors were excluded, and water availability was not limiting.

Identification of all insects collected (Tables 2, 5), excluding bees, was made by comparing our specimens with existing collections. Bee identifications except for *Apis*, *Parafidelia*, and *Tetraloniella* (provided by V. Whitehead, South African Museum, Cape Town) were made by C. D. Michener and R. W. Brooks, Snow Entomological Museum, Lawrence, Kansas. Insect voucher specimens are housed at the Snow Entomological Museum, Lawrence, Kansas, and the Natal Museum, Pietermaritzburg, South Africa.

Results

Inflorescence architecture

Most species of subgenus *Lapeirousia* have a simple or branched flowering stem, and each axis

Table 2. Analyses of pollen loads carried by insects collected on *Lapeirousia* species. *Prosoeca* = *Pr.*; *Tetraloniella* = *T.*; *Megistorhynchus* = *M.*; *Philoliche* = *Ph.*; *Parafidelia* = *Pa*; *Anthophora* = *A.*; *Heliotis* = *H.* Pollen types abbreviated as follows: Ac = *Acacia*; An = Anthericaceae; Co = Asteraceae; Gr = *Grielum*; He = *Hermannia*; La = *Lapeirousia*; Op = orchid pollinium; Pa = papilionoid legume (?*Wiborgia*); Pe = *Pelargonium*; Po = *Polygala*; Sc = Scrophulariaceae; Wa = mixed campanulales (?*Wahlenbergia*, *Prismatocarpus*); Ud = unidentified dicot; Um = unidentified monocot. Figures in parentheses after insect names indicate number of individuals. Figures in columns 3–16 are the numbers of insects bearing a particular pollen type.

Lapeirousia species	Insect taxon (n)	Pollen loads													
		Ac	An	Co	Gr	He	La	Op	Pa	Pe	Po	Sc	Wa	Ud	Um
		Subgenus *Lapeirousia*													
L. silenoides group															
L. dolomitica	Diptera														
	Pr. peringueyi (1)	—	—	—	—	—	1	—	—	—	—	—	—	1	—
L. jacquinii	Diptera														
	Pr. sp. (2)	—	—	—	—	—	2	—	—	—	—	—	1	1	1
	Pr. peringueyi (3)	—	—	—	—	—	3	—	2	—	—	—	—	—	—
L. oreogena	Diptera														
	Pr. sp. (5)	—	—	3	—	—	5	—	—	—	—	—	—	—	—
L. pyramidalis	Diptera														
subsp. *regalis*	*Pr. peringueyi* (5)	—	—	—	—	—	5	—	—	—	—	—	—	—	—
L. silenoides	Diptera														
	Pr. peringueyi (9)	—	—	—	—	—	9	—	—	8	—	—	—	—	1
L. violacea	Diptera														
	Pr. peringueyi (2)	—	—	1	—	—	2	—	—	—	—	—	—	—	—
L. divaricata group															
L. barklyi	Hymenoptra														
	Pa. major (1)	1	1	1	1	—	—	—	—	—	—	—	—	—	—
L. exilis	Diptera														
	Bombyliid (2)	—	—	—	—	—	2	—	—	—	—	—	—	—	—
	Hymenoptera														
	Apis mellifera (5)	—	—	—	—	—	5	—	—	—	—	—	—	—	—
L. divaricata	Lepidoptera														
	H. armigera (2)	—	—	2	—	—	1	—	—	—	1	—	—	—	—
	Hymenoptera														
	A. diversipes (5)	—	—	4	—	1	5	—	1	2	—	—	1	2	1
	A. krugeri (2)	—	—	1	—	2	2	—	—	1	—	—	—	—	—
	A. schulzei (1)	—	—	1	—	—	1	1	—	—	—	—	—	—	—
	A. braunsii (1)	—	—	—	—	1	1	—	—	—	—	—	—	—	—
	Amegilla grisella (1)	—	—	—	—	1	1	—	—	—	—	—	—	—	—
L. montana	Diptera														
	Bombyliid (2)	—	—	1	—	—	2	—	—	—	—	—	—	—	—
	Hymenoptera														
	A. krugeri (1)	—	—	1	—	1	1	—	—	—	—	—	—	—	—
	Lepidoptera														
	Cynthia cardui (1)	—	—	1	—	—	1	—	—	—	1	—	—	1	—
	H. armigera (2)	—	—	1	—	—	—	—	—	—	—	—	—	—	—
L. plicata	Hymenoptera														
	Apis mellifera (6)	—	—	—	—	—	6	—	—	—	—	—	—	—	—
	T. cf. *karroica* (4)	—	—	—	—	—	4	—	—	—	—	—	—	—	—
L. spinosa	Hymenoptera														
	Hoplitis similis (1)	—	—	1	—	—	1	—	—	—	—	—	—	—	—
L. fabricii group															
L. anceps	Diptera														
	M. longirostris (1)	—	—	1	—	—	1	—	—	1	—	—	1	1	—
L. fabricii	Diptera														
	Ph. gulosa (4)	—	1	1	—	—	4	—	—	2	1	—	—	1	—
	M. longirostris (3)	1	1	2	—	—	3	1	—	—	—	1	—	2	—

Table 2. Continued.

Lapeirousia species	Insect taxon (n)	Pollen loads													
		Ac	An	Co	Gr	He	La	Op	Pa	Pe	Po	Sc	Wa	Ud	Um
		Subgenus *Paniculata*													
L. azurea	Hymenoptera														
	A. diversipes (2)	—	—	2	—	1	2	1	—	1	—	—	1	2	1
L. fastigiata	Hymenoptera														
	A. diversipes (1)	1	—	—	—	—	1	—	—	—	—	—	—	1	—
	Hoplitis similis (1)	—	—	1	—	—	1	—	—	1	—	—	—	—	—
L. neglecta	Hymenoptera														
	Xylocopa hottentotta (1)	—	—	1	—	—	1	—	—	—	—	—	—	1	—

terminates in a spike of several to many flowers. In *L. oreogena, L. montana, L. plicata,* and *L. odoratissima* the inflorescence is a congested tuft of flowers borne at ground level. The number of flowers per plant varies considerably from year to year. Vigorous plants have as many as five spikes each and up to 20 flowers per tuft or spike.

FLORAL PHENOLOGY AND LIFE SPAN

Flowering in southern African species of subgenus *Lapeirousia* in the study area ranges from late May until early November (Fig. 1). Populations of each species bloom for two to four weeks. Exact timing of flowering within a species varies seasonally, and to some extent locally, depending on elevation and aspect. The higher the elevation the later a population comes into bloom. Despite considerable overlap in flowering times (Fig. 1), species may be characterized generally as early (June to mid August), mid (late August and September), or late season (October and November) bloomers.

The pattern of flower buds opening on an inflorescence is acropetal. In all species the perianth of a mature bud expands in the early to mid morning. The rate at which buds open and the longevity of the expanded perianth are dependent on ambient temperatures. The perianth of an open flower lives a maximum of five days if daily temperatures remain below 20°C and soils remain wet. At sunset the tepals of most species contract, partly or sometimes completely enclosing the exserted anthers and stigmas. In *L. exilis,* tropical African *L. odoratissima,* and *L. pyramidalis* (both subspecies) the tepals remain open all night.

The anthers of all species dehisce longitudinally within two to four hours after the tepals unfold. Pollen remains in the open thecae until contacted by insects or washed out by rain. The three stylar lobes do not normally expand to expose the receptive stigmas until the day after the anthers have dehisced. This suggests that all the species studied are weakly protandrous.

FLORAL MORPHOLOGY (FIG. 2)

In most species of subgenus *Lapeirousia* the perianth tube is cylindric. In *L. divaricata* and *L. spinosa,* however, the tube is funnel-shaped (Fig. 2H). In sixteen species the perianth tube always exceeds 17 mm in length, with a maximum of 76 mm in *L. anceps* (Table 5). In *L. divaricata, L. spinosa,* and *L. tenuis* the tube is less than 16 mm long. In *L. exilis, L. montana,* and *L. plicata,* however, the external length of the tube is misleading: the lower half of the tube is occluded by the style and intrusive ridges, decurrent on the filaments (Fig. 2K), so that the nectar is forced into the upper part of the tube. Functional tube length, in terms of accessibility of nectar to insects, is less than half what it appears to be (Table 5). Tube length is unusually variable in *L. anceps,* and may be as little as 20–30 mm in populations in the south of its range, and 45–76 mm in the west and north.

Seventeen species have a zygomorphic flower and unilateral, arcuate stamens and style (Table 5, Fig. 2A, E, H). In contrast, *L. oreogena, L. montana, L. plicata,* and *L. odoratissima* have actinomorphic flowers (Fig. 2J, K) in which the stamens and style are symmetrically disposed.

Tepals of the fully open flower either spread horizontally and are thus patent, or the dorsal (adaxial) tepal is erect (*Lapeirousia silenoides, L. pyramidalis, L. verecunda*), or inclined over the stamens (*L. divaricata, L. spinosa*). In species with zygomorphic flowers the dorsal tepal is broader and sometimes longer than the five other tepals. *Lapeirousia fabricii* and *L. barklyi* are the only species in which the tepals are broadly cupped below and patent above, and thus include the filaments. In several species with zygomorphic flowers (e.g., *L. divaricata, L. fa-*

Table 3. Nectar sugars in subgenus *Lapeirousia*. Nectar analyses for subgenus *Lapeirousia* were provided by B.-E. van Wyk, Rand Afrikaans University, Johannesburg, South Africa. Taxa of subgenus *Lapeirousia* are grouped according to flower type and pollinator class as in Table 2.

Species	Sample size n	Range of sugars % Fru	Glu	Suc	Sucrose/ (Fru + Glu)
L. silenoides group					
L. dolomitica					
subsp. *dolomitica*	2	4–9	12–14	77–84	3.35–5.25
subsp. *lewisiana*	4	5–8	14–19	74–81	2.85–4.26
	1	12	25	63	1.7
L. jacquinii	1	8	17	75	3.00
L. oreogena	4	11–17	19–25	58–69	1.38–2.26
L. pyramidalis					
subsp. *regalis*	3	8–12	14–17	74–81	2.45–3.55
	3	4–21	12–36	48–84	0.92–5.25
L. silenoides	3	5–8	18–27	65–77	1.86–3.35
L. violacea	1	9–13	15–16	71–76	2.45–3.17
L. divaricata group					
L. barklyi	1	36	40	24	0.32
L. divaricata	1	18	28	54	1.17
L. exilis	3	11–16	19–25	59–70	1.44–2.33
L. montana	3	6–24	16–30	46–88	0.85–4.00
L. plicata	2	15–16	21–31	54–63	1.17–1.70
L. pyramidalis					
subsp. *pyramidalis*	4	5–11	12–22	71–73	2.45–4.88
L. spinosa	1	13	20	67	2.03
L. fabricii group					
L. anceps	3	6–20	20–26	54–74	1.17–2.85
L. arenicola	4	5–11	10–18	71–85	2.45–5.67
L. fabricii	4	12–21	21–26	53–67	1.13–2.03
L. macrospatha	1	34	43	23	0.29
L. simulans	1	18	23	59	1.44
L. verecunda	2	8	18	74	2.85

bricii—Fig. 2H), each of the three lower tepals bears a raised ridge or narrow tooth. These appendages are absent in species with actinomorphic flowers.

In all species the stamens are equal in size, and the filaments are erect or sometimes slightly inclined. In zygomorphic-flowered species the anthers are parallel and face the center of the flower and the lower tepals and nectar guides, but in species with actinomorphic flowers the anthers are symmetrically disposed and face outward (Fig. 2J, K). The style branches of all species of subgenus *Lapeirousia* included in the study are divided for half their length. Although this is a generic characteristic, the style branches may be undivided in some species or populations of the genus (Goldblatt, 1972, 1990). The resulting six stylar arms, which spread outward and recurve, bear receptive stigmatic hairs that appear to be dry when observed under a 10× hand lens. The style arms are normally held between 1 and 3 mm above the anther apices, but in *L. arenicola, L. jacquinii, L. simulans,* and *L. violacea* the style arms become tangled in the anthers as the style lobes expand.

COMPATIBILITY AND FRUIT SET

We were unable to make a thorough study of compatibility in subgenus *Lapeirousia* but present the following observations. In *L. arenicola, L. jacquinii, L. simulans,* and *L. violacea,* stems cut at bud stage, maintained in water, and excluded from insects for two weeks routinely set full capsules containing the normal number of seeds for that species. Furthermore, *L. jacquinii* and *L. oreogena* have been noted to set seed in greenhouses where insect activity was excluded.

Lapeirousia barklyi, L. dolomitica subsp. *dolomi-*

Table 4. Nectar concentration and volume in subgenus *Lapeirousia*. Taxa of subgenus *Lapeirousia* are grouped according to flower type and pollinator class as in Table 2. Figures for nectar sugar concentration are means, standard deviation given in parentheses, but ranges are provided for less than three observations.

Species	Sample size	Nectar conc. (%)	Nectar volume (ml)
L. silenoides group			
L. dolomitica			
subsp. *dolomitica*	5	30.4 (2.4)	1.4–3.1
subsp. *lewisiana*	4	27.0 (0.8)	5.1–5.5
L. jacquinii	5	27.3 (2.9)	1.5–2.3
	1	25	1.5
L. oreogena	10	25.8 (1.6)	2.5–7.3
L. pyramidalis subsp. *regalis*	10	28.4 (2.8)	2.6–4.8
	3	28.2 (0.8)	2.6–4.8
L. silenoides	10	26.5 (1.1)	1.7–2.8
	3	29.0 (2.0)	2.3–3.6
	2	22–25	2.6–2.8
	1	26	2.1
L. violacea	3	25.0 (0)	1.5–1.6
	7	28.2 (2.9)	1.4–1.8
L. divaricata group			
L. barklyi	2	26–27.5	0.9–1.3
L. divaricata	7	26.7 (1.5)	0.6
L. exilis	10	34.1 (1.8)	0.9–1.7
L. montana	10	24.8 (2.2)	2.6–8.1
L. plicata	3	28.3 (3.2)	0.9–1.7
L. pyramidalis subsp. *pyramidalis*	3	30.1 (4.9)	0.7–1.7
	7	34.1 (1.6)	0.8–3.0
L. spinosa	2	31–32	1.1–1.2
L. tenuis	6	46.1 (5.1)	0.4–1.0
L. fabricii group			
L. anceps	10	23.5 (1.3)	1.5–3.1
	7	21.1 (1.4)	1.4–3.2
L. arenicola	4	32.8 (2.1)	0.6–0.7
L. fabricii	10	32.5 (2.3)	1.3–3.4
	5	24.8 (2.6)	0.8–1.5
	7	25.2 (1.1)	0.4–0.9
L. macrospatha	10	23.8 (1.5)	0.7–3.3
	3	21.0 (1.0)	3.5–5.7
L. simulans	4	25.8 (1.7)	0.8–1.5
L. verecunda	10	26.6 (2.5)	0.6–3.0

tica, L. pyramidalis, L. silenoides, and *L. spinosa* did not set fruit when inflorescences were maintained in water as in the species above. In these taxa dehisced anthers and stigmatic surfaces are in contact in both field populations and laboratory specimens. *Lapeirousia silenoides* and *L. pyramidalis* subsp. *regalis,* in which the anthers and stigmas are not in contact, do not set fruit even when self-pollinated by hand whether maintained in water or grown in pots. In field populations of *L. dolomitica, L. pyramidalis,* and *L. silenoides* individual plants were often observed to produce none, or only one or two mature capsules per spike after flowering.

FLORAL PRESENTATION, ATTRACTANTS, AND REWARDS

Field observations of species of subgenus *Lapeirousia* indicate that they can be divided into three morphological types based on floral characteristics (Tables 3, 4, 5), and that each of the floral types is associated with a different suite of pollinators. The three flower types are defined primarily by floral pigmentation and patterning in combination with perianth tube length. However, regardless of flower type, in zygomorphic flowers visible nectar guides are always confined to the three lower tepals (Fig.

Table 5. Floral characters and pollinators of *Lapeirousia* subg. *Lapeirousia* of Namaqualand and the Cape West Coast. *Prosoeca* and *Megistorhynchus* are long-proboscid flies of the family Nemestrinidae; *Philoliche* is a long-tongued fly of the family Tabanidae (only *P. gulosa* has been collected on species of subgenus *Lapeirousia*). Data here represent original observations; pollination of *L. oreogena* by *Prosoeca* sp. was first observed by K. Steiner (pers. comm.); pollination of *L. anceps* by *M. longirostris* was first reported by Hesse in Vogel (1954). Visits to *L. barklyi* by *Parafidelia major*, its presumed pollinator, have been noted by V. Whitehead (pers. comm.). Z = zygomorphic; A = actinomorphic; V = vivid red, purple, or violet shades with contrasting pale nectar guides; P = pale blue (or pink); W = white; CR = pale cream with red markings; N = no scent; S = weak odor; SS strong odor; — = no data.

	Flower					
Species	Symmetry	Color	Scent	Pollinator	Mouth part mm	Floral tube mm
L. silenoides group						
L. dolomitica						
subsp. *dolomitica*	Z	V	S	*Prosoeca peringueyi*	33	35–40
subsp. *lewisiana*	Z	V	N	—	—	45–50
L. jacquinii	Z	V	N	*Pr.* sp. and *Pr. peringueyi*	30	33–35
L. pyramidalis subsp. *regalis*	Z	V	N	*Pr. peringueyi*	35	40–47
L. oreogena	A	V	N	*Pr.* sp.	37	53–63(–70)
L. silenoides	Z	V	N	*Pr. peringueyi*	35	43–55
L. violacea	Z	V	N	*Pr. peringueyi*	32	34–40
L. divaricata group						
L. barklyi	Z	P	N	Parafidelia	—	
L. divaricata	Z	W	S	noctuid moth and anthophorid	7–8	23–25
				bees	8–10	9–11
L. exilis	A	W	SS	bombyliid flies and honey bees	6–8	20–25[1]
L. montana	A	P–W	S	various[2]	6–12	43–55[3]
L. plicata	A	W	N	bombyliid flies and honey bees	6–8	15–20[3]
L. pyramidalis subsp. *pyramidalis*	Z	P	SS	hawkmoth	22	25–28
L. spinosa	Z	W	S	*Hoplitis similis*	5	10–12
L. tenuis	Z	P	S	—	—	12–15
L. fabricii group						
L. anceps	Z	CR	N	*Megistorhynchus*	54–60	65–76
L. arenicola	Z	CR	N	—	—	17–21
L. fabricii	Z	CR	N	*Megitorhynchus*	42–46	38–47
				and *Philoliche*	31–34	45–48
L. macrospatha	Z	CR	N	—	—	31–34
L. simulans	Z	CR	N	—	—	28–35
L. verecunda	Z	CR	N	—	—	38–49

[1] A pocket of air in the base of the tube forces the nectar upwards ca. 8–10 mm, effective tube length 8–10 mm.
[2] Anthophorid bees (*Anthophora krugeri*), butterflies (*Cynthia cardui*), bombyliid flies, noctuid moths.
[3] Lower half of tube completely closed, effective tube length ca. 25 mm for *L. montana*, 5–8 mm for *L. plicata*.

2). In all species nectar is secreted at the base of the floral tube in volumes ranging from 20 to 76 ml (only 10–12 ml in *L. divaricata* and *L. spinosa*) (Table 4). In *L. exilis*, *L. montana*, and *L. plicata* occlusion of the basal part of the tube results in the nectar being forced into the upper half.

1. The *L. silenoides*-type (Fig. 2A): tepal pigmentation dark blue to purple or dark red, darker than the nectar guides; visible nectar guides yellow to white streaks or blotches near the tepal bases and sometimes a zone of intense pigmentation in the center of each lower tepal; callus ridges absent; no scent discernable to the human nose except in *L. dolomitica* subsp. *dolomitica*, which has a faint sweet odor; floral tubes 33–70 mm long; flowers actinomorphic or zygomorphic; nectar sucrose rich to sucrose dominant.

2. The *L. fabricii*-type (Fig. 2E): tepal pigmentation white to cream or pale pink (but usually pink to red on the reverse), lighter than the nectar guides; visible nectar guides dark red to maroon oblong to acute patterns at the tepal bases; callus

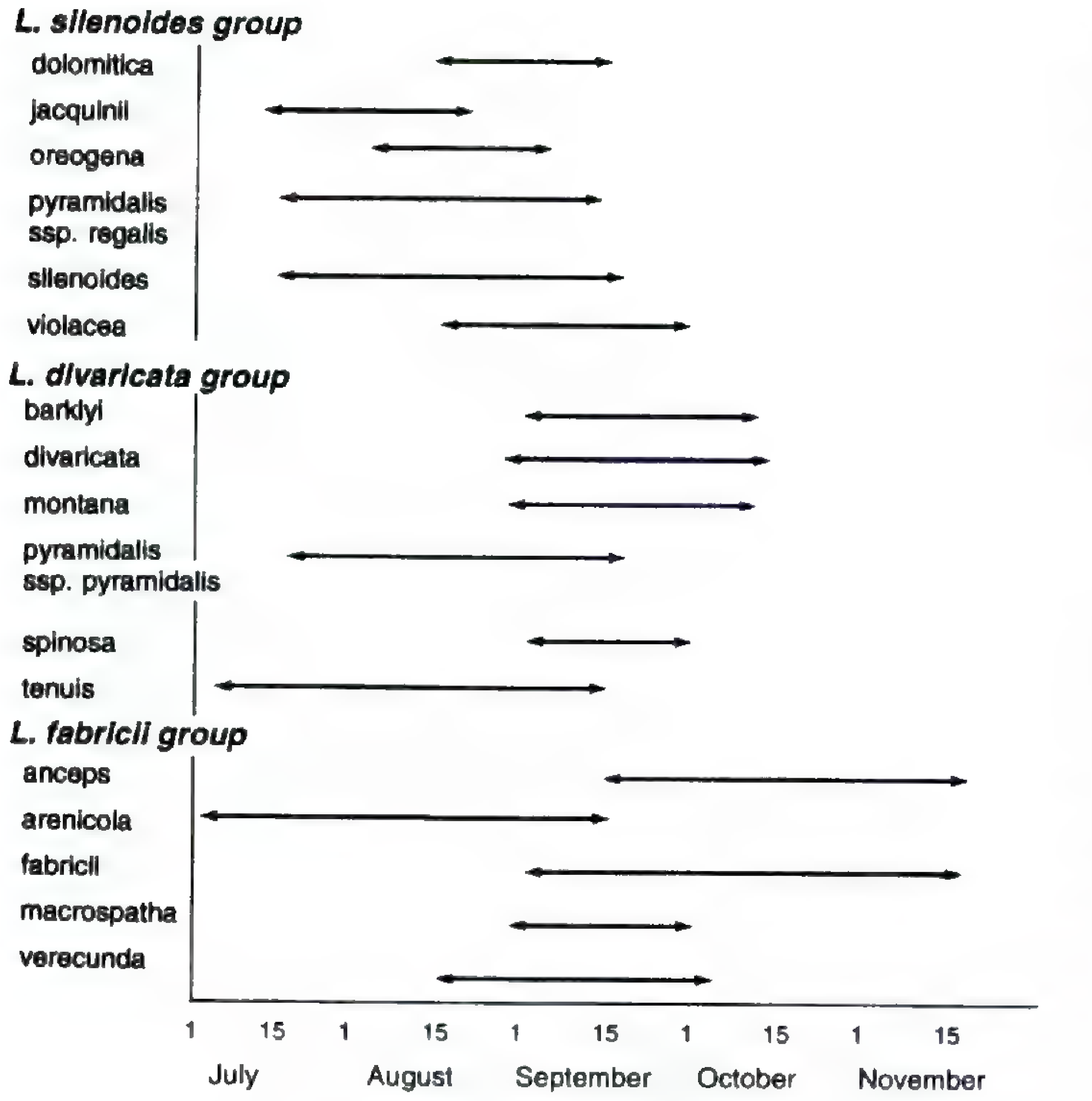

FIGURE 1. Flowering phenology of southern African species of subgenus *Lapeirousia* (i.e., excluding *L. odoratissima* and *L. littoralis* subsp. *caudata*). Species are arranged alphabetically in their respective floral guilds (Tables 2–5).

ridges usually present; no scent discernable to the human nose; floral tubes normally 30–76 mm long; flowers zygomorphic; nectar sucrose dominant or, in *L. macrospatha*, hexose rich.

3. The *L. divaricata*-type (Fig. 2H): tepal pigmentation white to cream or pale blue, lilac, or pink; visible nectar guides usually narrow to streaky, light purple sometimes grading to a yellow to cream-colored ridge at the base of the tepals or merely a white zone in the center of the flower (*L. montana*); scents present and variable, ranging from a cloyingly sweet heavy fragrance (*L. exilis*, *L. pyramidalis* subsp. *pyramidalis*) to a lightly sweet to mildly spicy scent reminiscent of *Dianthus* or *Narcissus*; callus ridges well developed in *L. divaricata*, *L. spinosa*, rare and inconspicuous in *L. pyramidalis* subsp. *pyramidalis*; floral tubes never exceeding 55 mm, in 3 of the 5 species tubes less than 30 mm (functional tube length always less than 35 mm); flowers actinomorphic or zygomorphic; nectar usually sucrose dominant, or sucrose rich (*L. montana*), or hexose rich (*L. barklyi*).

POLLINATION MECHANISMS

The three modes of floral presentation are accompanied by three different pollinator guilds. However, all floral foragers collected on *Lapeirousia* species had two foraging characters in common. First, pollen load analysis (Table 2) and observations of flight patterns indicate that all insects observed on *Lapeirousia* (Figs. 2, 3) forage actively on the flowers of some other co-blooming plants (Table 2). Second, no matter how elongated the mouth parts of the pollinator are, they are always somewhat shorter than the perianth tube (Table 5). In addition, an insect was rarely observed to probe anthers with its proboscis or actively collect pollen on any *Lapeirousia* species.

1. The *Lapeirousia silenoides* group. Nectar foraging in this flower type was dominated by long-tongued flies of the genus *Prosoeca* (Nemestrinidae) (Figs. 3A, B, 4A). *Prosoeca peringueyi* was observed foraging on the flowers of all species in this group (Tables 2, 5) excluding *L. oreogena*. *Lapeirousia oreogena* was visited by *Prosoeca* sp. nov. (Fig. 3B), and this fly species also visited *L. jacquinii* at two sites on the Nieuwoudtville Plateau (where *P. peringueyi* evidently does not occur). In both species of flies the insects hover while orienting and inserting their proboscis into the tube. When the proboscis has been fully inserted into a tube that is long enough to permit this, the insect grasps the tepals with its legs during the actual feeding process, but the wings remain in motion. By flexing the tibiae of the hind legs forward, the fly can use all three pairs of legs for grasping, and this is done in plants with both vertical and horizontal flower presentation. In flowers with short tubes that are visited for nectar, the body does not contact the stamens or stigmas so that pollination is not effected, and the fly hovers without grasping the tepals.

Prosoeca species are active on mild to warm days from mid morning to early afternoon, and again in the late afternoon. These flies remain at each *Lapeirousia* flower for 3–5 seconds, and pollen adheres to the frons and thorax in *L. oreogena* and on the dorsum and upper frons in other species. Often pollen depositions are so heavy they are visible to the naked eye. In species with zygomorphic flowers, the fly always orients itself in the same way, approaching the flower from the front, thus facing the anthers as it forages. Density of visitors varies considerably, ranging from four to five flies present locally at the same time, to as few as one or two flies at sites over periods as long as an hour. At one site, the top of Botterkloof Pass, where *L. jacquinii* and *L. violacea* co-occur, individuals of *P. peringueyi* were observed to visit both species indiscriminately. *Prosoeca* species typically interrupted foraging on *Lapeirousia* species to collect nectar on other species growing nearby, including *Pelargonium* species (Geraniaceae), and taxa of Iridaceae including *Babiana* species, *Sparaxis variegata* subsp. *metelerkampiae* (L. Bolus) Goldblatt, *Xenoscapa uliginosa* Goldblatt & Man-

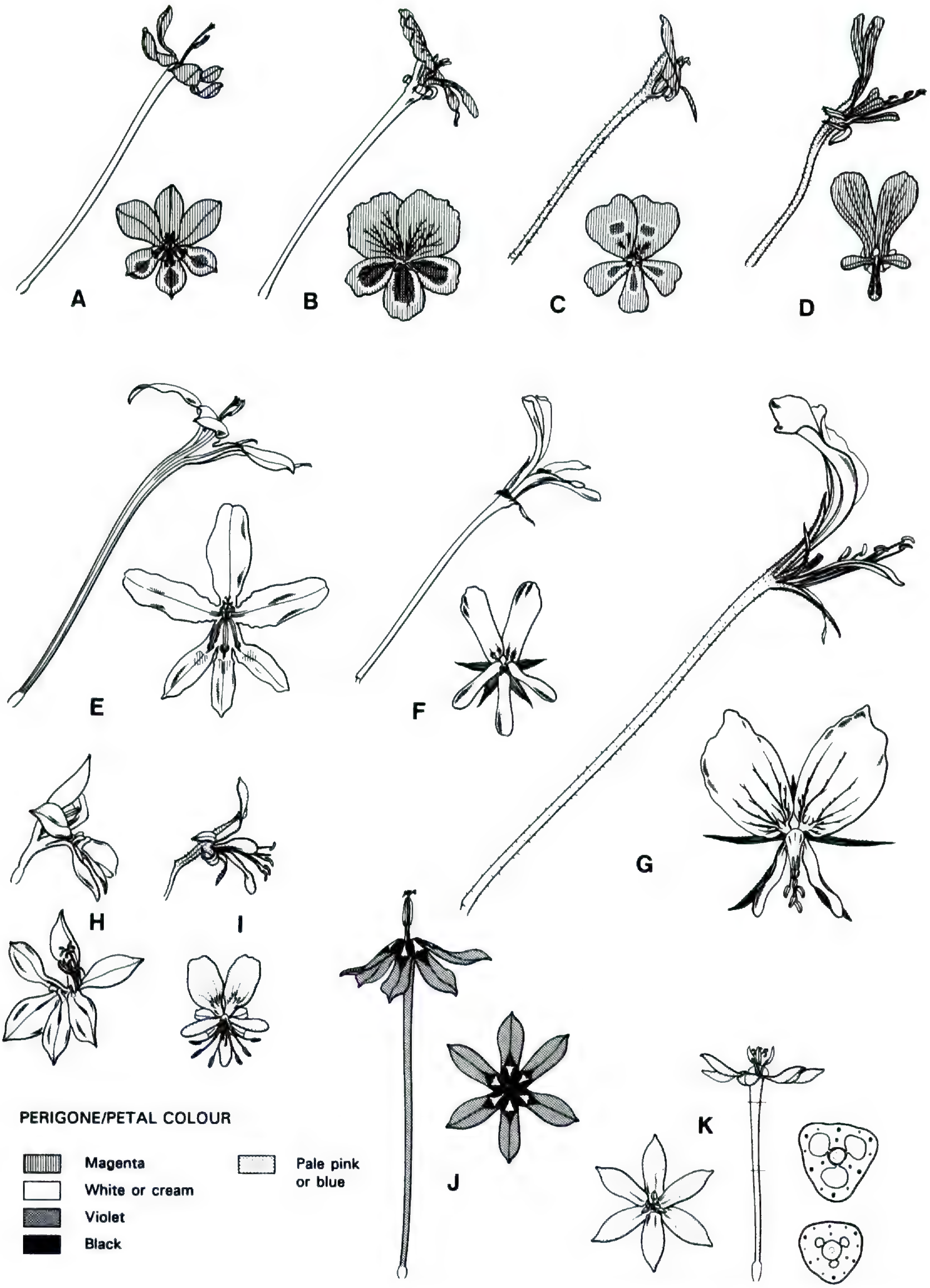

FIGURE 2. The main flower types in subgenus *Lapeirousia*, and selected flowers of other families resembling these flower types, which are visited by the same insects that are the primary pollinators of *Lapeirousia* species.—A. *L. silenoides*.—B. *Pelargonium sericifolium*.—C. *P. magenteum* J. J. A. Van der Walt.—D. *P. incrassatum* (Andr.) Sims.—E. *L. fabricii*.—F. *Pelargonium zonale* (L.) L'Hér. ex Sol.—G. *P. longicaule* (Jacq.) Harv.—H. *L. divaricata* (note prominent toothlike ridges on the lower tepals).—I. *Pelargonium scabrum* (L.) L'Herit.—J. *L. oreogena*.—K. *L. montana* (with transverse sections of the tube at different levels). Approximately ×⅔.

FIGURE 3.—A. *Prosoeca peringueyi* foraging on *Lapeirousia pyramidalis* subsp. *regalis.*—B. *Prosoeca* sp. nov. inserting its proboscis into the perianth tube of *L. oreogena.*—C. *Apis mellifera* foraging on *L. plicata.*—D. The noctuid moth *Heliotis armigera* grasping a flower of *L. divaricata.* Photographs were taken at study sites listed in Table 1.

ning, *Hesperantha latifolia* (Klatt) de Vos, and *Geissorhiza kamiesmontana* Goldblatt. Interestingly, the flowers of *Pelargonium sericifolium* van der Walt do not secrete nectar, although they have long floral tubes and may mimic flowers of species that do produce nectar (Fig. 2), suggesting pollination by deceit, a phenomenon best known in Orchidaceae.

Pollen deposition tends to be on the head and back part of the thorax (nototribic) in *Lapeirousia* except *L. oreogena*, but on the underside (sternotribic) in *Pelargonium*, a factor that reduces contamination of pollen loads and competition for vector sites on the insects' bodies.

2. The *L. fabricii* group. Nectar foraging in this group was limited to one species of fly in the Nemestrinidae (*Moegistorhynchus longirostris*—Fig. 4B) and one species of fly of the family Tabanidae (*Philoliche gulosa*—Fig. 4C). Both fly taxa are long-tongued and have mouth parts as long as or longer than the *Prosoeca* species discussed above. *Moegistorhynchus longirostris* was observed actively foraging on *L. fabricii* only in the late afternoon, 4:00–6:00 PM, at one study site in northern Namaqualand. The same fly species was recorded visiting *L. anceps* at the study site near Ysterfontein during the late morning on a cool and cloudy day.

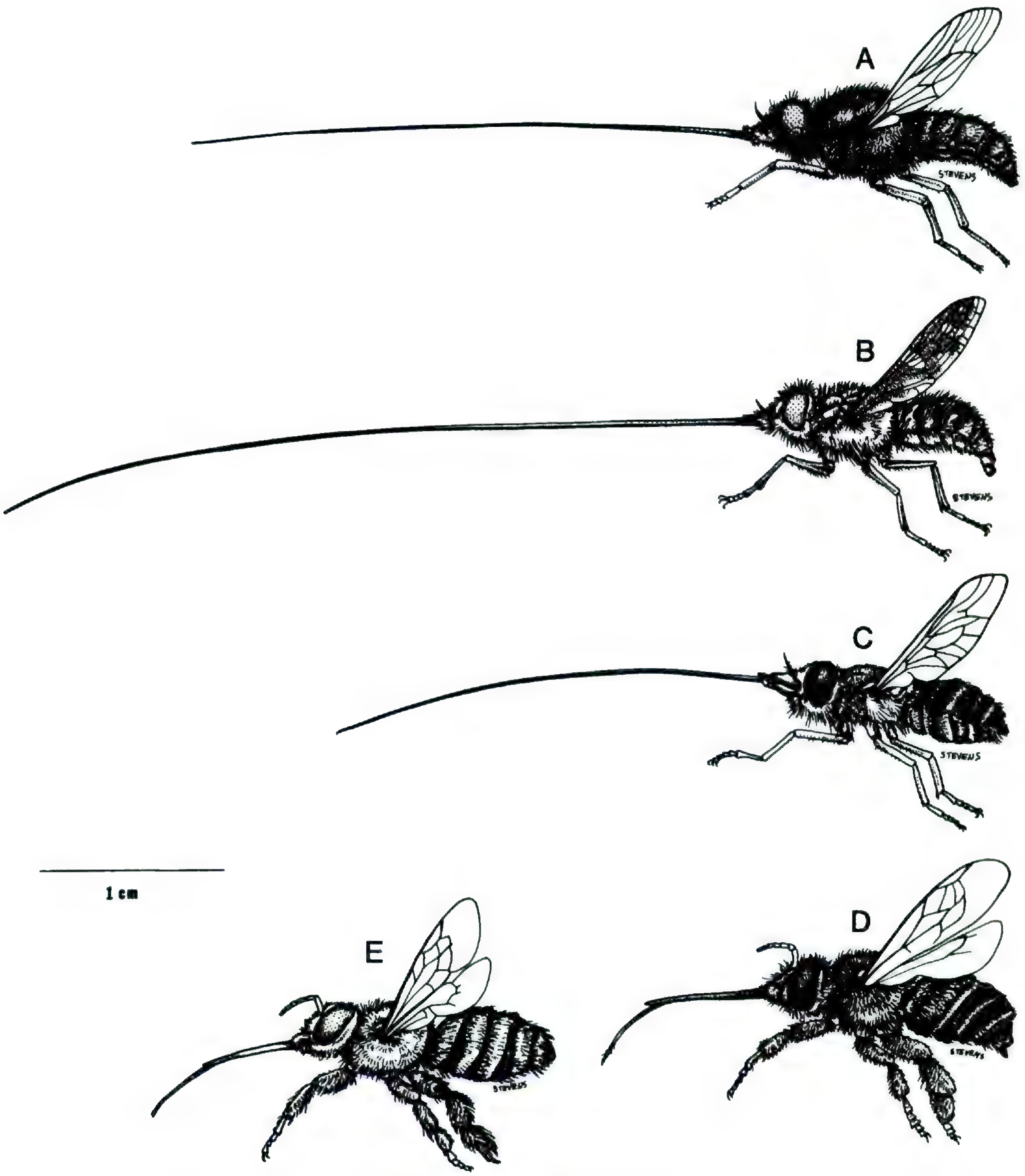

FIGURE 4. Primary pollinators of various species of subgenus *Lapeirousia*.—A. *Prosoeca peringueyi* (on *L. jacquinii*).—B. *Moegistorhynchus longirostris* (on *L. anceps*).—C. *Philoliche gulosa* (on *L. fabricii*).—D. *Anthophora diversipes* (on *L. divaricata*).—E. *Parafidelia major* (on *L. barklyi*). Scale bar = 10 mm.

Philoliche gulosa visited *Lapeirousia fabricii* at three sites between 11 AM and 3 PM. The foraging behavior of *Moegistorhynchus longirostris* and *P. gulosa* on *Lapeirousia* species was similar to the foraging behavior of *Prosoeca* species. Nemestrinid species fly with their mouth parts hanging downward, and forage for nectar by extending the fairly flexible proboscis either forward or downward depending on the characteristics of a particular flower. *Philoliche gulosa* flies with its proboscis extended forward, and its proboscis appears to be less flexible (S. Johnson, pers. comm.). *Moegistorhynchus longirostris* interrupted its foraging on *Lapeirousia* species to forage on the flowers of *Pelargonium* species, and species of two other Iridaceae, *Geissorhiza exscapa* (Thunb.) Goldblatt and *Babiana tubulosa* (Burm. f.) Ker-Gawl. We assume that *B. brachystachys* (Bak.) G. Lewis, *Ixia paniculata*

Delaroche, *Tritonia crispa* (L. f.) Ker-Gawl., *Geissorhiza confusa* Goldblatt, and *Gladiolus angustus* L. (all Iridaceae) also belong to this guild, because their flowers are similarly constructed and colored. *Moegistorhynchus* was also observed attempting to forage on the spurred but nectarless terrestrial orchid, *Disa draconis* (L. f.) Sw. The foraging of the fly on the orchid suggests another example of pollination by deceit, as the orchid produces creamy white flowers with narrow purple nectar guides, similar to floral presentation in *L. anceps*.

3. The *L. divaricata* group. Species in this group were visited by various combinations of Lepidoptera, bees, and bombyliid flies (Table 2). *Lapeirousia exilis* and *L. plicata* appear to be pollinated by a combination of bombyliid flies and Hymenoptera, including the native *Apis mellifera* (Apidae: Fig. 3C) and *Tetraloniella karooensis* (Anthophoridae). These insects were observed to contact anthers and stigmas while foraging, and pollen was brushed onto their bodies passively. All of the insects collected on flowers in this group have mouth parts shorter than *Philoliche gulosa* and the three taxa of nemestrinid flies (Table 5). Bees were active throughout most of the day, but were observed most frequently during the cooler periods before 11 AM or after 4 PM. The noctuid moth, *Heliothis armigera* (Fig. 3D), was observed in large numbers foraging on *L. divaricata* at two sites between 3:30 and 6 PM. Bombyliid flies, bees, *H. armigera*, and the butterfly, *Cynthia cardui* (Nymphalidae), did not usually forage by hovering. In zygomorphic flowers these insects always perched on the lower tepals before foraging for nectar.

The only insect observed to feed while hovering was the sphinx moth, *Hippotion celerio* (L.), which was seen but not captured in a population of *Lapeirousia pyramidalis* subsp. *pyramidalis* near de Wet (Worcester District). Pollen load analyses (Table 2) indicate that *H. armigera* and *Cynthia cardui* may be poor vectors of pollen compared to bees.

All bees collected on the *Lapeirousia divaricata* group belonged to long-tongued families. With the exception of two specimens of *Hoplitis similis* (Megachilidae) and one specimen of *Parafidelia* (Fideliidae), bees collected on the *L. divaricata* group belong in the families Anthophoridae and Apidae. Two anthophorid bee individuals collected (*Anthophora diversipes* and *Amegilla grisella*) were males, and the remainder, female. Analyses of pollen washes of the hairs on the hind legs and ventral abdominal region show two characteristics. First, all female bees collected on the *L. divaricata* group are polylectic and polytrophic. Field observations as well as pollen-load analyses showed that these bees collected pollen actively on nectarless flowers including *Cyanella alba* L. f. (Tecophilaeaceae) and *Hermannia* sp. (Sterculiaceae). *Hoplitis similis*, collected on *L. spinosa*, interrupted its visits to this species to forage on similarly pigmented *Moraea tortilis* Goldblatt, the flower of which is also a meranthium of the gullet type "blossoms" (see below), similar to that of *Iris*. The flowers of *M. tortilis* produce small quantities of nectar. Pollen-load analyses showed that all bees foraged on a much broader selection of taxa than were recorded for fly or moth specimens. Although female bees do not collect pollen actively on *Lapeirousia* flowers, the pollen deposited passively on the bee's head and thorax is combed off by the bee and deposited in the scopae. Bee activity in species of the *L. divaricata* group differed during the season. *Apis mellifera* was collected most frequently in July and early August, while the first anthophorids were not observed and netted until mid August.

Discussion

The limited published research on pollination ecology within the Iridaceae has tended to emphasize genera with meranthia of gullet flowers such as *Iris*, *Moraea*, and *Gynandriris* (Faegri & van der Pijl, 1979). The pollination ecology of *Lapeirousia*, however, is far closer to what has been described in the southern African genus *Nivenia* (Goldblatt & Bernhardt, 1990). In *Nivenia* floral tubes are not occluded and the stamens and styles are prominently displayed. The flowers are pollinated by long-tongued bees and nemestrinid flies in the genus *Prosoeca*. Access to the nectar-secreting tube is direct, and the insect head and thorax contact the anthers and stigma while the insect hovers or clings to the tepals.

The disparity between the length of the perianth tube in subgenus *Lapeirousia* and the shorter length of the mouth parts of the primary pollinators is quite easy to explain. Records of nectar secretion show that species in subgenus *Lapeirousia* secrete copious amounts of fluid for insect-pollinated flowers, and it is most unlikely that dominant pollinators are ever forced to extend their mouth parts to the base of the tube unless all the nectar has been removed by earlier foragers. More important, Darwin (1862) hypothesized that successful pollination of spurred orchids occurred when orchids evolved floral spurs slightly longer that the tongues of their pollinators, forcing the insect to ram its head down the floral throat, ensuring contact between the insect's head and the orchid's column. This has since been shown experimentally by Nilsson (1988). As

in the nectariferous orchids, species in subgenus *Lapeirousia* "oblige" their pollinators to make contact with the anthers and stigmatic surfaces of the style branches of the flowers that block or at least encircle the entrance to the floral tube.

Some members of subgenus *Lapeirousia* appear to be self-compatible. Spatial isolation between anthers and stigmas is not always well expressed in subgenus *Lapeirousia* and protandry is weakly developed. Four *Lapeirousia* species show successful fruit and seed production in the absence of pollinators. This is most likely the result of mechanical autogamy. (We consider the alternative possibility that apomixis takes place most unlikely: the phenomenon is unknown in Iridaceae.) Three of the four species that show self-pollination start flowering during the southern African winter, when rain and low temperatures may restrict pollinator activity. Mechanical autogamy by contact between the stigmatic surfaces and the pollen (or apomixis) then becomes a fail-safe mechanism in the absence of dependable pollinators. This has also been described in the late winter-early spring flowering herbs of North America (Schemske et al., 1978) and some terrestrial orchids of southern Australia (Dafni & Bernhardt, 1990).

There are two major differences between floral mechanisms in *Nivenia* versus *Lapeirousia*. First, fly pollination in *Nivenia* appears to be restricted to nemestrinids. In some *Lapeirousia* species pollination may be dependent on the long-tongued tabanid, *Philoliche gulosa*. Second, and more important, analysis of floral presentation and observation of floral foragers emphasize that pollination systems in southern African species of subgenus *Lapeirousia* can be subdivided into a minimum of three syndromes, or perhaps four if the sphinx moth syndrome is regarded as separate from the generalist system that otherwise prevails in those species with the *L. divaricata*-type flower.

Pollination by nectar-foraging flies has been treated as a relatively common but unspecialized syndrome in which many fly taxa visit the same flower, and the dispersal of pollen may be shared with co-foraging bees and butterflies (Grant & Grant, 1965; Barth, 1985). We may compare the more classical treatment of myophily with our results. Fly-pollination in *Lapeirousia* has evolved into such a specialized syndrome that two different modes of floral presentation appear to attract and depend on two different sets of fly genera. Species with the *L. silenoides*-type of flower appear to depend exclusively on two species of flies in one genus, *Prosoeca*. We also note that plants with the *L. silenoides*-type flower appear to be restricted to the west coast and adjacent near interior of southern Africa. The species exhibiting the *L. fabricii*-type of presentation frequently have marginally longer tubes than species that have the *L. silenoides*-type flower and seem to be pollinated exclusively by *Moegistorhynchus longirostris* and *Philoliche gulosa*. Plant species with this flower type occur widely across southern Africa, although they appear to be most frequent in the southwest and west of the subcontinent. Among the species with this type of flower, *L. anceps* stands out in its remarkable range of perianth tube lengths, 20–76 mm. The pattern suggests that only populations in the west and north of its range with tubes 45–76 mm long are pollinated by *M. longirostris*, because nectar is accessible to only this fly, given the length of its proboscis. Populations with shorter tubes, as little as 20–30 mm in the south of its range, cannot be pollinated by this fly species, which does not occur in this part of the range of *L. anceps*. The pollinator(s) for these short-tubed plants must be some other, presumably long-tongued fly, possibly *Philoliche gulosa*, which does occur here and has a proboscis 20–33 mm long. Evidently, tube length is extremely labile and may respond rapidly to selection by pollinators.

Despite the segregation of *Lapeirousia* species into three pollination guilds, the majority of species in this subgenus secrete sucrose-rich/sucrose-dominant nectar regardless of the major pollinators. This adds a new dimension to the analytic work and categorization of nectar by Baker & Baker (1983, 1990). In their earlier treatments of myophily the Bakers found that fly-pollinated flowers tended to be weak in sucrose, like the flowers pollinated by short-tongued bees. However, when the Bakers analyzed fly flowers they concentrated on taxa pollinated by short-tongued flies, such as the Muscidae, Syrphidae, and Phoridae. It now appears that just as flowers pollinated by long-tongued bees are usually rich in sucrose (Baker & Baker, 1983, 1990), flowers pollinated by long-tongued flies are also sucrose producers. Perhaps pollination by large-bodied, physically active insects that maintain wing movement while feeding (e.g., nemestrinids, *Philoliche*, sphinx moths and some anthophorids (Goldblatt & Bernhardt, 1990)) requires an emphasis on sucrose instead of hexose rewards that is independent of insect order.

Although certain *Lapeirousia* species may be pollinated by only one or two fly species, the degree of dependency in this insect-flower relationship is not shared to the same degree by the flies. As in so many proposed cases of co-adaptation, the *Lapeirousia* flowers appear to have become modified for pollination by specific flies to a greater extent than

flies have become modified for *Lapeirousia* flowers. The evidence for this unequal relationship is presented in the polytrophic foraging behavior of flies at field sites and confirmed by the results of pollen load analyses. It is more likely that the *Lapeirousia* species with *L. silenoides*-type and *L. fabricii*-type flowers belong to broader guilds, encouraging the partitioning of long-tongued fly pollination into more than one syndrome in the southern African flora. Observations on flowers in other genera and families visited by long-tongued flies, e.g., *Babiana* and *Hesperantha* (Iridaceae), *Pelargonium* (Geraniaceae), suggest that floral presentation for pollination by *Prosoeca* species or by *Moegistorhynchus* and *Philoliche* shows a high degree of convergence in such floral characters as color patterns and tube or spur length. The types of floral presentation in southern African plants pollinated by different fly genera and species may ultimately prove to be as diverse as, yet distinct from, pollination guilds in other parts of the world, such as members of the neotropical flora that are pollinated by straight-billed hummingbirds versus those plant taxa pollinated by hermit hummingbirds with curved bills (Feinsinger & Colwell, 1978; Feinsinger et al., 1985).

Pollen-load analysis of the bee-pollinated members of the *Lapeirousia divaricata* group suggests community dynamics typical of flowers pollinated by long-tongued bees in other parts of the world. These anthophorids and long-tongued bees in the Apidae (e.g., *Bombus, Euglossa*) often show foraging strategies in which individual bees balance visits to nectarless flowers (e.g., *Dianella* (Phormiaceae), *Echeandia* (Anthericaceae), *Schrankia* (Fabaceae), *Acacia* (Fabaceae), *Hibbertia* (Dilleniaceae)) that offer copious pollen with visits to plants that produce copious nectar, but from which pollen is not collected actively (Bernhardt, 1989, 1990; Bernhardt & Montalvo, 1979; Bernhardt, 1995). The presence of the pollen of nectarless *Cyanella* and *Hermannia* on female anthophorids collected on flowers of the *L. divaricata*-type suggests that community pollination by long-tongued bees in southern Africa may not be significantly different from those syndromes cited above for the floras of Central America and southern Australia, and the woodlands and prairies of North America (Schemske et al., 1978).

Members of subgenus *Lapeirousia* now join an expanding list of plant taxa in which segregated pollen flow is due in part to ethological isolation (Grant, 1994). In subgenus *Lapeirousia* ethological isolation (sensu Grant, 1994) appears to be based on two factors. First, as in *Aquilegia* (Ranunculaceae), different pollinators may be restricted to different plant taxa as a partial consequence of mechanical isolation (Grant, 1971), as bees and moths are probably unable to forage successfully on the flowers of *Lapeirousia* species in which tube length far exceeds tongue length. Second, ethological isolation must also be based in part on flower constancy as differing modes of floral presentation, featuring diverse color patterns and scent production, produce different responses in the foraging behavior of local pollinators that are polytrophic and/or polylectic. That is, flies and bees are not expected to respond to the same forms of floral advertisement due to their different visual and olfactory senses (Barth, 1985).

Observations of interspecific hybridization in sympatric and co-blooming populations of *Lapeirousia fabricii* and *L. jacquinii*, and of *L. silenoides* and *L. verecunda*, respectively, indicate that when members of the different pollination guilds are sympatric, ethological mechanisms are sometimes insufficient to prevent interspecific pollination, as evidenced by the recent discovery of scattered hybrids between members of each of the above pairs. In fact, species belonging to different guilds seldom co-occur, and when they do they usually flower at different times. These observations suggest that floral divergence is not a result of selection for prepollination isolating mechanisms.

Postpollination isolating mechanisms may operate in *Lapeirousia jacquinii* and *L. violacea*. These species belong to the same guild and have been seen to be visited by the same fly individuals. Despite this, no F1 hybrids have been found after three years of fieldwork, and it seems likely that biochemical recognition and rejection of interspecific pollens may be a more important form of interspecific isolation within some *Lapeirousia* species belonging to the same pollination guild. However, in other instances interspecific pollen recognition may offer incomplete isolation, as *L. jacquinii* and *L. pyramidalis* subsp. *regalis* have the same mode of floral presentation and hybrids between the two have been recorded at one site (Goldblatt & Manning, unpublished).

Information on pollination systems in subgenus *Lapeirousia* may now be combined with a cladistic analysis to help determine the evolution of pollination syndromes, extensively discussed by Goldblatt & Manning (in mss.). The picture that emerges from that study (Fig. 5) indicates most strongly that the dependence of a *Lapeirousia* species on a particular guild of long-tongued pollinators has originated several times. While it is true that some sister species (e.g., *L. dolomitica* and *L. violacea* or *L.*

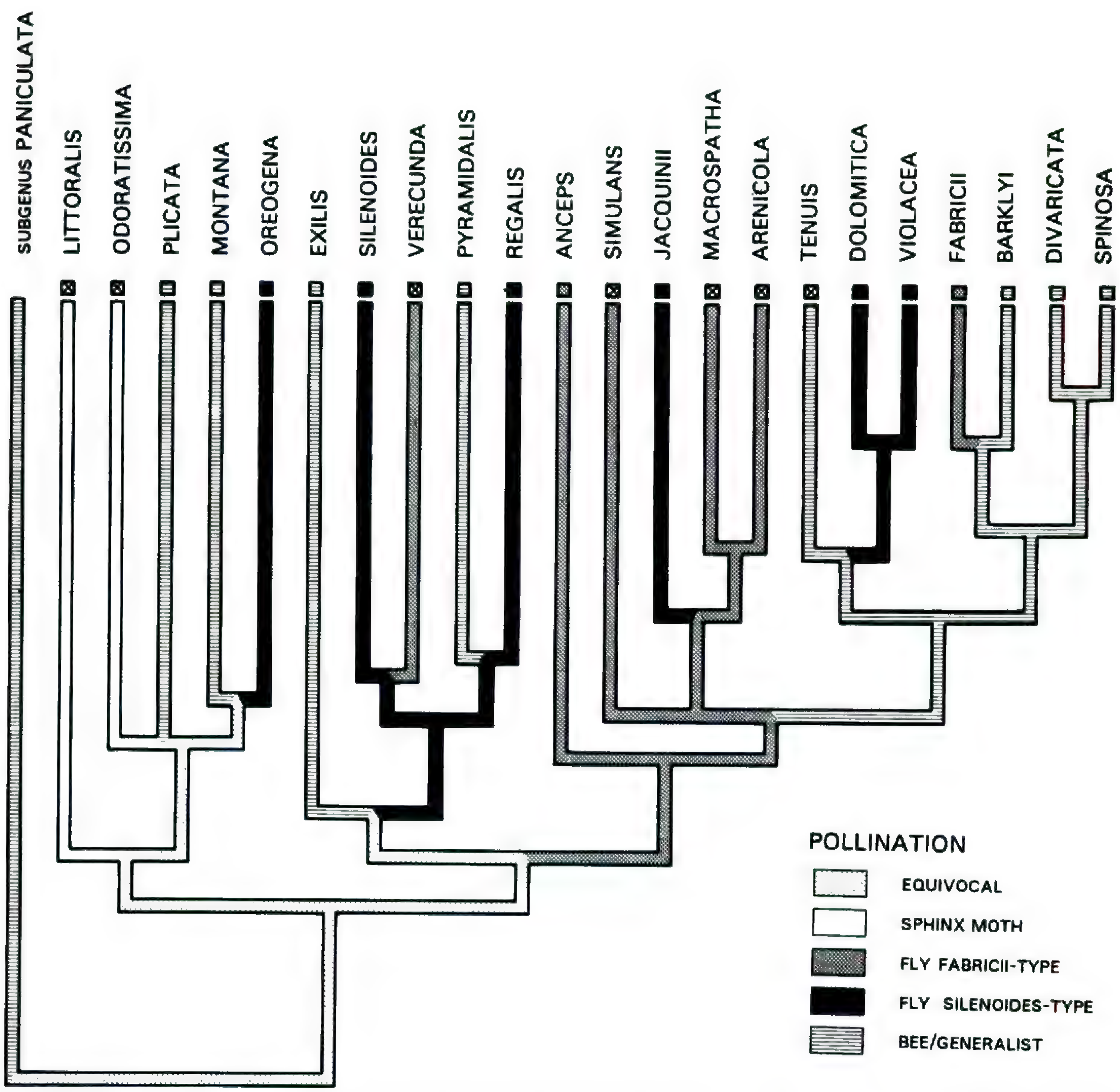

FIGURE 5. Phylogenetic tree showing putative relationships of the species of subgenus *Lapeirousia* (from Goldblatt & Manning, in mss.) with pollination syndromes mapped on the branches. The cross in the block below the species name indicates pollinator(s) inferred from floral morphology. The tree is one of four equally parsimonious trees obtained using the Hennig86 package of programs for cladistic analysis (Farris, 1988), after successive weighting, consensus index (C.I.) 81, retention index (R.I.) 91. The initial analysis using the mh* and bb* options yielded 1332 equally parsimonious trees, length 74, C.I. 48, R.I. 67. The four final trees differed in the position of only two terminal taxa and do not affect our conclusion that the two long-tongued fly pollination systems evolved independently several times in subgenus *Lapeirousia*. The details of the cladistic analysis are presented in detail in a separate paper (Goldblatt & Manning, in mss.).

divaricata and *L. spinosa*) share the same pollination syndrome, this sharing appears to be an exception within the current phylogenetic tree. From the combined data we conclude that floral evolution in subgenus *Lapeirousia* is extremely labile and probably reflects a rapid response to the relative diversity of potential vectors within a given geographic area.

It is possible to predict with some degree of confidence the ancestral mode of pollination within subgenus *Lapeirousia*. We know that in the outgroup, subgenus *Paniculata*, the majority of species bear relatively short-tubed flowers with pollination types most similar to the *L. divaricata*-type in subgenus *Lapeirousia*. Goldblatt (1990) reported that two species, *L. erythrantha* (Klatt) Bak. and *L. avasmontana* Dinter (both tropical members of subgenus *Paniculata*), are actively visited by bees,

wasps, and diurnal Lepidoptera, and *L. sandersonii* Bak. of the subgenus is visited predominantly by diurnal Lepidoptera (Manning, unpublished). This is consistent with our observations here (Table 2) for *L. azurea* (Eckl. ex Bak.) Goldblatt, *L. fastigiata* (Lam.) Ker-Gawl., and *L. neglecta* Goldblatt & Manning, and Scott Elliot's (1891) report for *L. corymbosa* (all subgenus *Paniculata*), also most commonly visited by bees. Therefore, short-tubed and funnel-shaped flowers dependent on Hymenoptera and Lepidoptera may be basal to subgenus *Lapeirousia*, and it seems far more parsimonious to infer that flowers with long perianth tubes and associated nectar guides are ultimately derived from short-tubed flowers with simple nectar guides. In light of this, the terminal position of the species pair, *L. divaricata-L. spinosa*, primarily pollinated by bees, and nested within a clade of long-tongued fly-pollinated taxa, evidently represents a reversal to an ancestral pollination strategy. This emphasizes the extreme degree of adaptive radiation exhibited within subgenus *Lapeirousia* and the impact of pollinators on floral morphology and biochemistry.

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REVIEW OF THE GENUS *STREPHONEMA* (COMBRETACEAE)[1]

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ABSTRACT

The genus *Strephonema* (Combretaceae) comprises three species restricted to the wet tropical forests of West Africa (*S. pseudocola*) and Central Africa (*S. mannii* and *S. sericeum*). A possible fourth species from Central Africa cannot as yet be distinguished for want of flowers. The presence of a simple receptacle that cannot be divided into a distinct upper and lower part, and of a large fruit with the remnants of the flower located near the base rather than at the top, makes it possible to distinguish this genus from all other Combretaceae. *Strephonema* species are small to dominant forest-trees; *S. mannii* is only known from regular inundated forests. A key to the species is presented, along with brief descriptions and complete synonymy. Several lectotypes are designated.

Although the genus *Strephonema* comprises only three species, preparing the treatment for the *Flore du Gabon* was at times a challenging task. In particular, material placed here tentatively under *S. sericeum* is very variable in indumentum and leaf shape, and it is difficult to distinguish species when there are no flowers. Fruits were sometimes available, but there is little variation in shape and size of the fruits among the species.

Morphologically, *Strephonema* exhibits more putatively primitive characters than any other genus in the family. All *Strephonema* flowers are bisexual. The receptacle does not close completely around the ovary as in all other Combretaceae; instead, the ovary is placed on the inside and at the bottom of the more or less cup-shaped receptacle, thus making it visible from outside. This means that if the remnants of the calyx, petals, and stamens persist in fruit they are located near its base rather than at the top of the fruit, as in all other genera. Furthermore, *Strephonema* fruits are not provided with wings or other dispersal adaptations common elsewhere in Combretaceae.

Strephonema was first described in 1867 as a new genus of Lythraceae, but Engler & Diels (1900) clearly placed it in Combretaceae. The most comprehensive taxonomic works on *Strephonema* to date are the treatments for the Floras of Zaire and Cameroun by Liben (1968, 1983). In both cases Liben incorrectly applied the name *S. pseudocola* to some of the fruiting or sterile collections from these areas. This error was copied by Vivien & Faure (1985: 174, 175). *Strephonema pseudocola* was described from Ivory Coast, and it is the only species of this genus growing west of Nigeria, easily recognized by flowers that are much larger than those of the other species. In Cameroun and Gabon several flowering collections have been made of *S. mannii* and *S. sericeum* but none of *S. pseudocola*. For this reason and because the other species are better known today, one of the conclusions of this publication is that *S. pseudocola* does not occur east of Ghana. The specimens from central Africa that were formerly believed to belong to *S. pseudocola* are now identified as *S. mannii* or *S. sericeum*.

SYSTEMATICS

Strephonema Hook. f., in Benth. & Hook., Gen. Pl. 1: 782. 1867. TYPE: *Strephonema mannii* Hook. f. (lectotype, designated here).

Large or small trees. Leaves alternate, simple and entire, often with glands on the margins (glands next to the petiole stalked), venation pinnate, at least the upper main lateral nerves anastomosing a few millimeters from the leaf margin, tertiary veins semi-parallel and almost perpendicular to the midrib. Flowers 5-merous, with a distinct pedicel. Receptacle broadly infundibuliform to campanulate. Petals ciliate but otherwise glabrous. Stamens 10, bi-seriate; filaments glabrous; anthers often septate. One semi-superior ovary with two ovules and one style. Fruit one-seeded, resembling a large gall, subglobose, sessile, succulent, glabrous, receptacle usually still evident on the base of the fruit; seed with two succulent and ± planoconvex cotyledons, without endosperm. Seedling with hypogeal germi-

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nation, cotyledons remaining in the fruit, first leaves scalelike.

A genus of three or possibly four species restricted to humid tropical Africa.

KEY TO THE SPECIES OF *STREPHONEMA*

1a. Petals 5–7 mm long; in West Africa from Guinea to Ghana .... *S. pseudocola*
1b. Petals 3–4 mm long; in Nigeria and Central Africa.
   2a. Inflorescence and flowers almost glabrous, young leaves inconspicuously hairy; inflorescence rachis always shorter than 2.5 cm and usually ca. 1 cm long; tree always growing in marshy places .... *S. mannii*
   2b. Inflorescence, flowers, and young leaves conspicuously hairy; inflorescence rachis usually much longer than 2.5 cm; trees rarely growing in marshy places .... *S. sericeum*

Strephonema mannii Hook. f., in Benth. & Hook., Gen. Pl. 1: 782. 1867. TYPE: Nigeria. Old Calabar (fl. Feb.), *Mann 2293* (lectotype, designated here, K; fragment: BR).

Strephonema tessmannii Mildbraed, Engl. Bot. Jahrb. 51: 357. 1914. TYPE: Cameroun. Campo, Bebai, road to Olanga (fl Oct.), *Tessmann 779* (syntype, B destroyed; lectotype, designated here, K); Akonangi, road to Ngun (fl. Apr.), *Tessmann 999* (syntype, B destroyed).

Strephonema tessmannii Mildbraed var. *micranthum* Mildbraed, Engl. Bot. Jahrb. 51: 358. 1914. TYPE: Cameroun. Campo, Bebai, road to Nsesong (fl. Feb.), *Tessmann 843* (holotype, B destroyed). NEOTYPE: Cameroun. Just E of Kribi (fl. June), *Bos 4895* (neotype, WAG; isoneotypes, BR, K, MO, P).

Tree up to 30 m high, trunk to 20 cm diam., sometimes with buttresses or buttress-shaped stiltroots. Leaves: petiole up to 2 cm long; blade oblong-elliptic to oblong-obovate, up to 30 × 9 cm, coriaceous, glabrous, often with glands on the margin; young leaves wine-red, glabrous at first sight but with many very small two-armed hairs beneath; 7–15 pairs of main lateral nerves; apex acuminate. Inflorescence in the leaf-axil or subcauliflorous, subumbellate, rachis up to 2.5 cm long but usually much smaller, almost glabrous. Bracts 1–2 mm long, persistent. Pedicel 4–7 mm long. Receptacle ca. 3 mm high and 4 mm diam., almost glabrous, pale green to brown-green. Calyx lobes ciliate. Petals orbicular, 3–4 mm diam., ciliate with very small hairs, otherwise glabrous, white. Stamens exserted for ca. 4 mm; anthers ca. 1.5 mm long, without septa, pale yellow. Style exserted for ca. 5 mm, white. Fruit up to 7 cm diam., brownish, pustulate.

Additional specimens examined. NIGERIA. Along Calabar–Atimbo road, 4°57′N, 8°22′E (fl Sep.), *Daramola et al. FHI 76937* (K); Oyi River banks (ster.), *Latilo FHI 30952* (K); Eket, 4°39′N, 7°55′E (fl), *Talbot s.n.* (BM). CAMEROUN. Kumbe Dene Waterfall (fl Feb.), *Babute 18* (FHO); Mbouge bush (fl Feb.), *Babute 20* (FHO); Bityé, Dja River 50 km ENE of Sangmélima (fl), *Bates 993* (BM), (fl), *Bates 1726* (P); Ebolowa (fl), *Bates 1968* (BM, BR, MO); 7.5 km S of Kribi (fl Nov.), *Bos 3193* (BR, K, MO, P, WAG); 8 km from Kribi, 2°54′N, 9°57′E (fl Nov.), *Bos 3396* (BR, K, MO, P, WAG); 4–5 km E of Kribi (fl Oct.), *Bos 5449* (BR, MO, P, WAG); 5 km SE of Kribi (fl, fr. juv. Mar.), *W. de Wilde et al. 2098* (WAG); Takamanda Forest Reserve, near Mbilishe, 6°13′N, 9°24′E (fr Apr.), *Doumenge 414* (MO); Tissongo, Douala-Edea Forest Reserve (fl July), *Eartlan 1977–2* (E, K); near Douala (fr June), *Fleury* in *Chevalier 33185* (P); Edoup, Yaoundé region (ster.), *Fleury* in *Chevalier 33365* (P); 20 km SE of Douala (fr Aug.), *Leeuwenberg 6479* (MO, P, WAG); 5 km NE of Douala, 4°04′N, 9°43′E (fl May), *Letouzey 14936* (BR, K, P); Ndian River, 20 km SW of Mundemba (fl June), *Letouzey 15159* (BR, P); (Victoria =) Limbe, 4°01′N, 9°12′E (fl May), *Maitland 1145* (K); Tissongo (fr Aug.), *McKey & Eartlan 174* (K); Mbu Bakundu, Mamfe road, 5°02′N, 9°19′E (fr Jan.), *Nemba & Mambo 713* (MO); Mivini, 33 km W of Nyabessan, ca. 2°30′N, 10°15′E (fl Dec.), *Nkongmeneck 451* (BR); Douala Edea Reserve, near Lake Tissongo (fl Jan.), *Thomas 289* (K); Korup Reserve, Ndium River bank (fl Apr.), *Thomas 362* (K); banks of the Ndian River, between Bulu and Last Banana, 4°53′N, 8°47′E (fl July), *Thomas 2348* (P, WAG); near Mundemba, 4°57′N, 8°54′E (ster.), *Thomas 6806* (MO); Douala Edea Reserve, Lake Tissongo (ster.), *Waterman & McKey 820* (K). GABON. Rabi area, ca. 1°55′S, 9°50′E (fl Mar.), *Breteler et al. 9439* (WAG), (fl, y fr Mar.), *Breteler et al. 9518* (WAG), (fl Apr.), *Breteler et al. 9707* (WAG); Rabi area, Lake Divangui (fr Oct.), *Breteler et al. 10266* (WAG); near Libreville, ca. 0°23′N, 9°27′E (fl May), *Fleury* in *Chevalier 33690* (P); Ivindo River, Chutes du Kongué, 0°20′N, 12°42′E (fl. bud Dec.), *Hladik 1896* (P); Dighaba (fl Jan.), *Le Testu 2317* (P); Bitam, 2°07′N, 11°28′E (fl Mar.), *Le Testu 9024* (BM, BR, HBG, P, WAG), (fl Mar.), *Le Testu 9038* (BM, BR, P); Evorombil, 2°10′N, 12°05′E (fl Aug.), *Le Testu 9250* (BM, BR, MO, P); Oyem, 1°37′N, 11°36′E (fl June), *Le Testu 9607* (HBG, K, MO, P); Minvoul, 2°09′N, 12°07′E (fl. bud Oct.), *Louis 1919* (WAG); Gabon River (fl), *Mann 945* (P); Ofoubou River bassin (fl), *Reitsma 3754* (WAG); bassin (ster.), *Reitsma OF 2–48* (WAG); Fernan Faz, 1°35′S, 9°17′E (fr Oct.), *Walker 95* (P, WAG); Minkebé, ca. 1°30′N, 12°49′E (ster.), *Wilks E 115* (WAG). CONGO. Zanaga Distr. (ster.), *Sita 3217* (IEC); Zanaga Distr., near Bikolo and Kimbembé aux rives Lulali, ca. 2°50′S, 13°50′E (ster.), *Sita 3241* (IEC).

Ecology. Riverine and swamp forest in rainforest areas, up to 600 m altitude. Flowering and fruiting throughout the year.

Note. This species is very uniform throughout its geographic range. It can be locally common to dominant in swamp forest.

Strephonema pseudocola A. Chevalier, Bull. Soc. Bot. France 58: 172. 1912. TYPE: Ivory Coast. Cavally Valley, Prolo, 4°26′N, 7°32′W (fl Aug.), *Chevalier 19870* (lectotype, designated here, P).

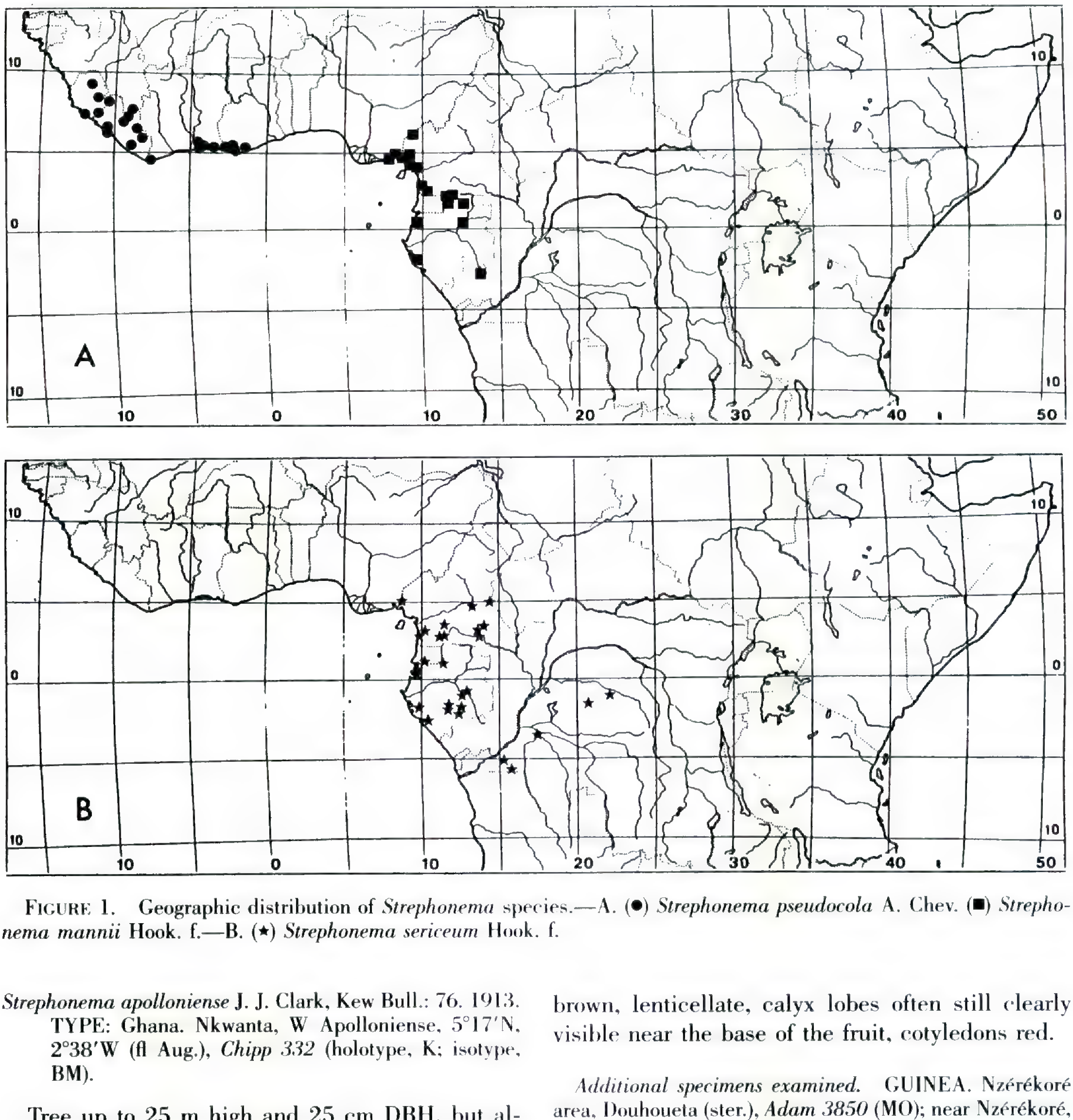

FIGURE 1. Geographic distribution of *Strephonema* species.—A. (●) *Strephonema pseudocola* A. Chev. (■) *Strephonema mannii* Hook. f.—B. (★) *Strephonema sericeum* Hook. f.

Strephonema apolloniense J. J. Clark, Kew Bull.: 76. 1913. TYPE: Ghana. Nkwanta, W Apolloniense, 5°17′N, 2°38′W (fl Aug.), *Chipp 332* (holotype, K; isotype, BM).

Tree up to 25 m high and 25 cm DBH, but already flowering when 4 m high. Bark gray. Exudate clear and slimy, appearing some time after slashing. Leaves: petiole up to 1.5 cm long; blade oblong-obovate, up to 40 × 12 cm, glabrous or locally with remnants of indumentum, often with glands on the margin; 8–18 pairs of main lateral nerves; very young leaves totally covered with two-armed hairs; apex acuminate. Inflorescence scarcely branched, rachis up to 12 cm long, covered with two-armed hairs. Bracts 3–4 mm long. Pedicel 2–6 mm long. Receptacle 6–8 mm high and 5–7 mm wide, pubescent, brown-green. Calyx lobes 2–3 mm long. Petals obovate, 5–7 × 3–4 mm, ciliate along the margin, white. Stamens exserted for ca. 10 mm; filaments white; anthers oblong-ovate in outline, 1.5–2 mm long, with many septa, yellow. Style exserted for ca. 15 mm. Fruit 4–6.5 × 3–4.5 × 2.5–4 cm, brown, lenticellate, calyx lobes often still clearly visible near the base of the fruit, cotyledons red.

Additional specimens examined. GUINEA. Nzérékoré area, Douhoueta (ster.), *Adam 3850* (MO); near Nzérékoré, ca. 7°45′N, 8°49′W (fl Sep.), *Jacques-Felix 1111* (P). SIERRA LEONE. Mt Loma (ster.), *Adam 22842* (MO); Baiima (fl Sep.), *Deighton 3055* (BR, K, P); Nimini S Forest Reserve, ca. 8°30′N, 11°08′W (fl bud Aug.), *Fox 168* (K); Loma Mts., near Nama Dji (seedling), *Jeager 1846* (P, WAG); between Kondembaya and Kauda, ca. 9°23′N, 11°34′W (ster.) *Jaeger 7510* (P); Kambui Forest Reserve, Waanje Valley, ca. 7°18′N, 12°08′W (ster.) *King 52* (K); Bo–Bumpe road, ca. 5 mi. from Bo (fr) *Morton & Jarr SL 1696* (GC, WAG); Nimini S Forest Reserve (fl Aug.) *Samai 370* (K); Kambui Hill Reserve, ca. 7°35′N, 11°06′W (ster.) *Wallace 113* (FHO). LIBERIA. Mt. Nimba (fl Oct.) *Adam 25046* (MO); Mèkepa area, Granfield (ster.), *Adam 25165* (MO); between Nimba and Yéképa, ca. 7°32′N, 8°33′E (fl Sep.) *Adam 26145* (MO, P); Mt. Nimba (ster.), *Adam 28561* (MO); Tchien area, Pine town (fr Nov.), *Adam 30315* (MO); Browntown, Tchien (fl Aug.), *Baldwin 7083* (K); Tchien Distr., Monroviatown (fl Aug.), *Baldwin 8007* (K, MO); Tapeta, 6°29′N, 8°51′E (fl Aug.), *Baldwin 9116* (K); Karmadhun = Kamatahum, ca. 8°13′N, 10°14′W (fl Nov.), *Baldwin 10203* (K); Grand Cape Mount Co.: Mecca,

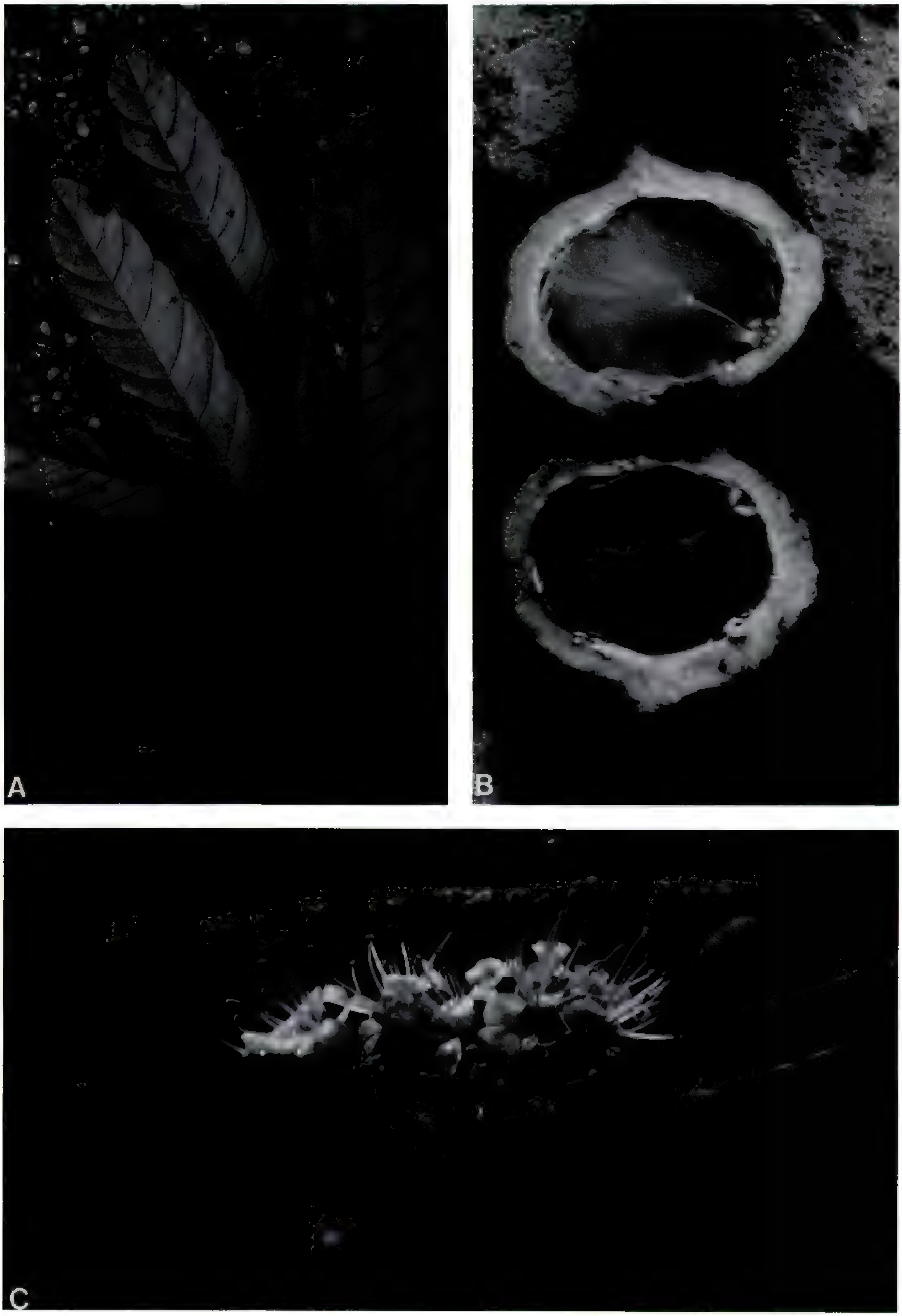

FIGURE 2.—A. *Strephonema sericeum* Hook. f., leaves. B, C. *Strephonema mannii* Hook. f.—B. Cross section of fruit, showing the two planoconvex cotyledons.—C. Inflorescence.

FIGURE 3. A, B, C. *Strephonema mannii* Hook. f.—A. Fruit showing the remnants of the receptacle located around the stipe.—B. Inflorescence with flower buds and one open flower.—C. Young fruits with calyx lobes still green and clearly visible.

6°43′N, 10°39′W (ster.), *Baldwin 10831* (K); Lofa River (fr Dec.), *Baldwin 10947* (K); Dukwia River, ca. 6°14′N, 10°32′W (fr Nov.), *Cooper 121* (BM, FHO, K); Ganta, 7°14′N, 8°59′W (fl Oct.), *Harley 1020* (K); Kitoma, 7°19′N, 8°46′W (fr), *Harley 1124* (K, WAG); Tapeta (fl July), *Jansen 905* (WAG); Gbarnga, 7°00′N, 9°29′W (fl Sep.), *Linder 767* (K, P); Tchien, 6°04′N, 8°08′W (fr juv. Oct.), *Versteegh & Jansen 808* (BR, MO, WAG). IVORY COAST. Banco Forest (ster.), *Aké Assi 13752* (K); Abidjan (ster.), *Aubréville 61* (BR, FHO, K, P), (fl) *Aubréville 128*

(BR, K, P); Boto (fr Mar.), *Aubréville 2060* (P); Teké forest, 5°33′N, 4°03′W (fr Feb.), *Bamps 2064* (BR, K, P, WAG); Bébasso Forest (fr Feb.), *Bamps 2437* (BR, K); region Bingerville, Abidjan, Dabou, ca. 5°20′N, 4°20′W (fl), *Chevalier 15235* (P), (fl) *Chevalier 15454* (P), (fl) *Chevalier 15524* (P), (fl) *Chevalier 15570* (P); Agnéby Valley, Mbago (fr Feb.) *Chevalier 16189* (P); Alipé (ster.) *Chevalier 17485* (P); 3 km N of Sikenssi, 5°40′N, 4°34′W (fr Jan.) *de Koning 137* (WAG); Banco Forest NP (fl Aug.) *de Koning 2069* (WAG), (fr Nov.) *de Koning 2808* (WAG), (fr Dec.) *de Koning 2897* (WAG); cult. ORSTOM (seedling), *de Koning 3530* (WAG), (seedling) *de Koning 3800* (WAG), (seedling) *de Koning 5660* (WAG); Banco Forest (fr), *de Koning 6443* (WAG); km 30 on new road Abidjan-Ndouci, 5°28′N, 4°14′W (fl Aug.) *de Kruif 311* (WAG); Anguédedou forest, near Abidjan (fl Sep.) *J. de Wilde 291* (WAG); Banco Forest NP (fr Jan.) *de Wit 9055* (WAG); M. Vizios concession (fr Dec.) *Fleury* in *Chevalier 33043* (P); Banco (fl Jan.) *Martineau 313* (P); between Aboisso and Bonoua, ca. 5°22′N, 3°24′W (fl Sep.), *Thijssen 283* (WAG); 20 km W of Abidjan (fl Aug.) *Versteegh & den Outer 667* (WAG). GHANA. Kakum Forest Reserve, ca. 5°25′N, 1°20′W (fl bud July), *Andoh 5736* (BM, BR, FHO); Neung Forest Reserve, ca. 5°08′N, 2°00′W (fr Jan.), *Enti Sp 500* (BR, MO); Aiyinasi to Mpataba road (fr Dec.), *Enti & Hall GC 36151* (FHO, GC); Simpa (ster.), *Vigne 2772* (FHO); near Prestea, ca. 5°26′N, 2°09′W (fr Sep.) *Vigne 3090* (FHO, GC).

Ecology. Rainforest. Flowering and fruiting between July and February.

Note. When he published *S. pseudocola*, Chevalier selected two types, one for the flowers and one for the fruits. Here the flowering syntype is selected for the lectotype.

Strephonema sericeum Hook. f., in Benth. & Hook., Gen. Pl. 1: 782. 1867. TYPE: Equatorial Guinea. Muni River (fl Aug.), *Mann 1753* (holotype, K; isotype, P).

Strephonema polybotryum Mildbraed, Engl. Bot. Jahrb. 51: 356. 1914. TYPE: Cameroun. 22 km E of Ebolowa, 2°56′N, 11°22′E (fl June), *Mildbraed 5645* (holotype, B destroyed; lectotype, designated here, HBG; fragment, BR).

Strephonema gilletii De Wildeman, Bull. Brux. 8: 119, tab. 3: figs. 1–5. 1923. TYPE: Zaire. Botanical garden of Kisantu (fl bud May), *Gillet s.n.* (holotype, BR; isotype, P).

Strephonema klaineana Pierre, nomen on drawing of *Klaine 288 = Pierre 6423*, ca. anno 1900.

Tree up to 45 m high and 350 cm diam., often with 5–6 buttresses ca. 1 m high. Bark brown-red, without exudate, up to 10 mm in diam. Leaves: petiole up to 2 cm long; blade elliptic to oblong-obovate, 5.5–25 × 2–9 cm, papery to coriaceous, glabrous or locally with appressed two-armed hairs beneath, margin often reflexed, with or without glands on the margin; young leaves reddish, totally covered with appressed two-armed hairs; 5–17 pairs of main lateral nerves, sometimes hardly visible; apex acuminate to long acuminate. Inflorescence branched, rachis up to 5 cm long, appressed pubescent. Bracts ca. 3 mm long, often early caducous. Pedicel 2–10 mm long. Receptacle 3–5 mm high and 4–5 mm wide, hairy. Petals obovate, 3–4 × 2–3 mm, ciliate along the margin with long hairs otherwise glabrous, white. Stamens exserted for 4–5 mm; filaments white; anthers 0.5–1 mm long, septate or not, yellow. Style exserted for ca. 7 mm, greenish white. Fruit up to 7 cm diam., irregular globular, brown warty.

Additional specimens examined. CAMEROUN. 6 km from Kribi, 2°54′N, 9°57′E (fr Nov.), *Bos 3358* (WAG); Hill above N'Kolandom, 2°48′N, 11°10′E (fl Nov.), *J. de Wilde 7713* (WAG); close to N'Kolandom (fl Apr.), *J. de Wilde 8161* (WAG); near Mbalmayo, ca. 3°31′N, 11°30′E (ster. Apr.), *Letouzey SRFK 1277* (P); S of Dimako, ca. 5°02′N, 14°23′E (ster.), *Letouzey 2671* (P); Nika, 4°36′N, 13°13′E (ster. Jan.), *Letouzey 2843* (P); just NW of Eschienbot, 3°08′N, 13°46′E (ster.), *Letouzey 3682* (P); near Bella, 45 km NE of Kribi, ca. 3°10′N, 10°10′E (ster. Jan.), *Letouzey 4154* (P); between Mpan and Badekok, 50 km E of Lomie, ca. 3°19′N, 14°01′E (ster. Aug.), *Letouzey 5618* (P); Dja River, 5 km WSW of Zoulabot 2, 2°53′N, 13°52′E (ster.), *Letouzey 11913* (P); Ebolowa (fl), *Mildbraed 7629* (K); Exploitation forestière de Dimako (fl bud), *Rérolle 10* (P); Korup NP, 5°01′N, 8°50′E (fr juv. Mar.), *Thomas 4585* (MO); Lombe Study area, Tissongo, Douala-Edea Forest Reserve, ca. 3°35′N, 9°54′E (fr June), *Waterman & McKey 827* (E, K). EQUATORIAL GUINEA. 3 km ESE of Okuamkos, 1°05′N, 10°10′E (ster.), *Wilks 1723* (WAG), (fl June) *Wilks 1725* (WAG). GABON. Sibang (fl Sep.), *Bernard SRF 267* (P); ca. 30 km ENE of Lastoursville, ca. 0°40′S, 13°00′E (ster.), *Breteler et al. 10620* (WAG); Rabi-Kounga River area (ster.), *Breteler et al. 11502* (WAG), (ster. incl. wood sample) *Breteler et al. 11552* (WAG); 18 km E of Libreville (fl Aug.), *De Saint Aubin s.n.* (P); 24 km SE of Cocobeach, ca. 0°45′N, 9°20′E (ster. Aug.), *De Saint Aubin SRF 1939* (P); near Libreville, ca. 0°23′N, 9°27′E (fl July), *Klaine 53* (BR, K, MO, P, Z), (fl Aug.) *Klaine 165* (HBG, WAG), (fl July) *Klaine 258* (BR, E, K, P), (fl July) *Klaine 288 = Pierre 6423* (BM, BR, E, K, P), (fl) *Klaine 329* (BR, HBG, K, P), (fl, fr July) *Klaine 533* (P), (fl July) *Klaine 1009* (P), (fl Aug.) *Klaine 1305* (BR, P), (fl) *Klaine 1915* (P, WAG), (ster.) *Klaine 2649* (P), (fl July) *Klaine 2967* (E, K, P), (fl July) *Klaine 2987* (BM, K, P); UFO-Noyah (ster. Apr.), *Lachiver SRF 1099* (P); Pagha, 1°34′S, 10°42′E (fl Nov.), *Le Testu 5095* (BM, BR, MO, P, WAG); Bandi (fl Oct.), *Le Testu 5570* (BM, BR, P); Benguémamba, 2°09′S, 12°18′E (fl June), *Le Testu 6522* (BM, BR, P); Ndenga, 1°55′S, 11°47′E (fl July), *Le Testu 6538* (BM, BR, MO, P, WAG); Sacamicanda, 1°02′S, 12°45′E (fl Dec.), *Le Testu 7731* (K, MO, P); Minguembé, 1°45′S, 12°27′E (fl Apr.), *Le Testu 8052* (BM, BR, MO, P, WAG), (fl Apr.) *Le Testu 8054* (BM, BR, P, WAG); Banguidjoumi, 1°35′S, 12°33′E (fl Apr.) *Le Testu 8056* (BM, BR, P, WAG); Moucwala, 1°24′S, 12°14′E (fl Aug.), *Le Testu 8222* (BM, BR, P, WAG); Ngango, 1°36′S, 12°25′E (fl May), *Le Testu 8808* (BM, BR, K, P); Ngwa, 0°59′N, 11°20′E (fl May), *Le Testu 9585* (BM, BR, P, HBG, WAG); Akia River bassin, affluent de Muni River (ster.), *Morel SRF 58* (P); Atem–Ayong (ster.), *Reitsma CO-12–14* (WAG), (ster.) *Reitsma CO-15–11* (WAG); Doudou Mts., ca. 30 km SW of Doussala, 2°32′S, 10°29′E (fr juv. Feb.),

Reitsma 1965 (MA, MO, WAG); Sibange (fl Aug.), *Soyaux 110* (K, P, Z); N of Rabi–Kounga, 1°47′S, 9°50′E (fl May), *Wieringa & Nzabi 1046* (WAG). ZAIRE. Ekwyelo (Bandundu), ca. 3°18′S, 17°21′E (fr Apr.), *Bolema 2886* (BR); Kisantu garden, venant marais de Boko Mfumu Disu, 5°02′S, 15°10′E (ster.), *Callens 2041* (BR); Bokota-Boseka, ca. 1°00′S, 22°08′E (fl bud), *Evrard 5660* (BR); Likete, 0°43′S, 21°24′E (fr June), *Ghesquiere 2762* (BR); s.l. (kiemplant) *Gillet s.n.* (BR); Kisantu, 5°08′S, 15°06′E (fr Oct.) *Louis 13304* (BR); Kilongo, forêt mar. (ster.), *Pauwels 470* (BR); Popokabaka Terr., Nto Mbomba, forêt marécageuse, 5°43′S, 15°51′E (ster.), *Pauwels 3974* (BR).

Ecology. Rainforest up to 600 m altitude. Flowering and fruiting season probably different across its range.

Note. *Strephonema sericeum* is very variable in the indumentum of the inflorescence and flowers and in the shape of the leaves. However, attempts to recognize taxonomic entities on the basis of these differences were unsuccessful.

The wide variation in the leaf shape of material previously placed in *S. polybotryum* has caused much confusion. Letouzey collected many specimens of this species in Cameroun with variously shaped leaves, of which the largest are many times larger than the smallest. He mentioned, on notes in the Paris herbarium, as a possible cause for the these differences that the tree probably grows different sized leaves in different seasons. This idea is supported by the fact that almost all fertile collections have small leaves only.

The differences between *S. gilletti* and material incorrectly attributed to *S. pseudocola* by Liben (1968) in the Flore du Congo, were primarily based on leaf-shape and nervation, a difference that is not tenable. A collection made by Ghesquiere (*Ghesquiere 2762*), includes the two types of leaves from a single tree.

INCOMPLETELY KNOWN MATERIAL

Strephonema spec. 1 (Jongkind); *Strephonema* spec. 1, Liben, Combretaceae. Flore du Congo du Rwanda et du Burundi: 4. 1968.

Flowers still unknown, leaf characters different from material of *S. sericeum* from Zaire.

Specimens examined. Zaire. Bokutu, ca. 0°15′N, 20°45′E (fr Sep.) *Dubois 530* (BR); Baringa on the Maringa, 0°45′N, 20°52′E (fr Oct.) *Evrard 5089* (BR).

Note. Without flowers it is not possible to assess with confidence whether this material is distinct from *S. sericeum.*

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ADDITIONS TO THE ACANTHACEAE OF PANAMA[1]

Thomas F. Daniel[2] *and Lucinda A. McDade*[3]

Abstract

Nine species of Acanthaceae are added to the known flora of Panama, including the newly described species *Aphelandra kuna*. Range extensions into Panama are reported for *A. scolnikae, Justicia ephemera, Odontonema cuspidatum, O. rubrum, Sanchezia lutea,* and *S. parvibracteata.* Revised keys to all Panamanian species of *Aphelandra, Odontonema,* and *Sanchezia* are provided. Two cultivated species, *Pachystachys lutea* and *Justicia betonica,* are also recorded from Panama for the first time.

The treatment of Acanthaceae for the *Flora of Panama* (Durkee, 1978) is now 17 years old. It serves as an extremely helpful facilitator for recognizing additions to the acanthaceous flora of Panama. Most of the new taxa and distributional records discovered since 1978 have been in regions remote from the well-collected provinces of central Panama. Darién Province and the Comarca de San Blas in eastern Panama (adjacent to Colombia) and the Caribbean slope of western Panama (which is becoming increasingly accessible via new roads) have yielded most of the additions of Acanthaceae to the Panamanian flora. Further discoveries are to be expected in these regions as they become more thoroughly explored. On the other hand, because of abundant "geographic taxonomy" in the Neotropics, some species undoubtedly will be combined with others as more collecting and study make geographic patterns apparent.

In this paper, we add nine species to the Acanthaceae known from Panama. These include the description of a new species of *Aphelandra*; documentation and discussion of six species in four genera that are newly reported from Panama; and notes on the occurrence of two species that are cultivated in Panama. We also provide revised keys to all Panamanian species of *Aphelandra, Odontonema,* and *Sanchezia.*

A New Species of *Aphelandra*

About 175 species are currently recognized in the wholly neotropical genus *Aphelandra* (Wasshausen, 1975; McDade, 1984; Daniel, 1991). The updated checklist for Panama (D'Arcy, 1987) included a number of nomenclatural changes and new taxa that had been recognized in the genus since Durkee's (1978) publication. D'Arcy (1987) listed a total of 19 species. McDade (1984) has argued that two of these do not actually occur in Panama. The two Panamanian specimens cited by Durkee (1978) as *A. crenata* Leonard (otherwise known only from South America) are readily referable to *A. campanensis* Durkee (*Wedel 2501*) and *A. hartwegiana* Nees (*Allen 5094*). These three species are part of a lineage with branched pedunculate inflorescences, obtuse bracts, falcate bracteoles, and distinctive pollen (see McDade, 1984); the Central American taxa should be viewed as provisional pending revision of the entire group. Durkee (1978) identified a number of plants from the ridges of the low, central Panamanian ranges as *A. pilosa* Leonard. *Aphelandra pilosa* is a South American species with profusely branching inflorescences, short bracts (5–6 mm), and relatively short corollas (ca. 60 mm). It is unlike any *Aphelandra* that occur in Central America. On the basis of morphology and experimental hybridizations, McDade (1984) argued that specimens treated by Durkee (1978) as *A. pilosa* are likely to be hybrids between *A. sinclairiana* Nees and *A. gracilis* Leonard. With the removal of *A. crenata* and *A. pilosa* and the additions (see below) of a new species and a geographic range extension, there are a total of 19 species of *Aphelandra* in Panama and one putative hybrid.

Aphelandra kuna T. F. Daniel & McDade, sp. nov. TYPE: Panama. Panamá: El Llano—Cartí Hwy., ca. 12 km N of El Llano, 19 July 1974,

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[1] We are grateful to S. Myers for rendering the line drawing, D. Ubick for SEM assistance, and the curators of herbaria cited in the text for loans. Daniel's fieldwork in Panama was generously facilitated by G. McPherson and H. Herrera. McDade's fieldwork was supported by the National Geographic Society (grant to W. Starnes).

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R. Dressler 4667 (holotype, US; isotype, DUKE). Figure 1.

Herbae perennes usque ad 1.3 m altae. Folia opposita, petiolata, laminae ellipticae vel obovato-ellipticae, 125–240 mm longae, 48–91 mm latae, 2.2–2.9-plo longiores quam latiores. Spicae dense bracteatae, 60–100 mm longae. Bracteae subroseae, ovatae vel ligulatae, erectae, (26–)30–49 mm longae, 10–17 mm latae, margine dentatae dentibus utroque latere 3–4. Corolla atropurpurea, 43–45 mm longa, extus dense sericea. Stamina inclusa, 4.5–5.5 mm longa, thecae 2.8–3.5 mm longae, apice pubescentes.

Monocaulous perennial herbs to 1.3 m tall. Young stems subquadrate (often somewhat collapsed in dried specimens) or flattened, sparsely pubescent with appressed eglandular trichomes to 1 mm long or nearly glabrate. Leaves opposite, petiolate, petioles to 50 mm long, blades elliptic to obovate-elliptic, 125–240 mm long, 48–91 mm wide, 2.2–2.9 times longer than wide, gradually attenuate to decurrent along petiole at base, acuminate at apex, the surfaces sparsely pubescent with appressed eglandular trichomes or becoming glabrate on adaxial surface. Inflorescence a terminal, sessile to short-pedunculate (peduncles to 5 mm long), densely bracteate spike, 60–100 mm long (excluding flowers), 32–45 mm wide (excluding flowers) near midspike, rachis pubescent with antrorsely appressed eglandular trichomes 0.4–1 mm long (sericeous). Bracts pinkish green to pinkish purple, ovate to strap-shaped, erect, (26–)30–49 mm long, 10–17 mm wide, attenuate to long-attenuate at apex, abaxial surface sericeous with eglandular trichomes, lacking nectariferous glands, margin conspicuously dentate (except for proximalmost bracts) with 3–4 teeth per side, the teeth 1.5–7 mm long. Bracteoles lance-subulate, strongly conduplicate, 19–31 mm long, 1.6–3 mm wide, pubescence similar to bracts. Calyx 5-lobed, 8–10 mm long, lobes lanceolate to subulate, unequal in size (i.e., one conspicuously longer and wider than others, the remaining lobes subequal to unequal in size), 6–9.7 mm long, 0.4–1 mm wide, abaxial surface sparsely pubescent to nearly glabrous. Corolla dark purplish with white on lower lip, 43–45 mm long, externally densely sericeous (especially the tube), upper lip 8–10 mm long, bilobed with rounded to acute lobes 3.5–5.5 mm long, 1.5–2.5 mm wide, lower lip 11–12 mm long, deeply trilobed, lateral lobes linear-elliptic, 8.5–11.5 mm long, 2.5–4 mm wide, lower central lobe elliptic, 9–12 mm long, 4.2–7.2 mm wide. Stamens inserted in distal ⅓ of corolla tube, included, 4.5–5.5 mm long, thecae 2.8–3.5 mm long, apically pubescent; staminode 1 mm long. Style 32 mm long, stigma asymmetrically funnelform, 1 mm long. Capsule and seeds not seen.

Distribution and habitat. Known only from the vicinity of the type locality in northeastern Panama (Panamá and Comarca de San Blas), in the Serranía de San Blas; plants occur along streams in a region of tropical wet forest at elevations from 300 to 400 m.

Phenology. Flowering: June–July, September, November.

Paratypes. PANAMA. **Panamá:** El Llano–Cartí Rd., 10–12 km from El Llano, 12 Nov. 1974, *P. Maas & R. Dressler 1706* (U, US). **Comarca de San Blas:** El Llano–Cartí Rd., 18 km from Interamerican Hwy., 9°19′N, 78°55′W, 7 Sep. 1984, *G. de Nevers 3876* (CAS); Km 16.7 of El Llano–Cartí Rd., 9°19′N, 78°55′W, 16 June 1985, *G. de Nevers 5906* (DUKE).

Two of the known collections of *Aphelandra kuna* were identified as *A. tridentata* Hemsl., a species known only from Costa Rica that bears a superficial resemblance. Using keys to species of *Aphelandra* in southern Central America (Durkee, 1978, 1986), one might mistake *A. kuna* for either *A. tridentata* or *A. aurantiaca* (Scheidw.) Lindl. It differs from the former by its pinkish (vs. green) bracts with 3–4 (vs. 1–2) teeth per side, 19–31 (vs. 11–12) mm long bracteoles that are strongly (vs. slightly, if at all) conduplicate, purplish (vs. red) corollas that are externally densely sericeous (vs. pubescent with flexuose trichomes) and have a deeply (vs. shallowly) trilobed lower lip with lobes 8.5–11.5 (vs. up to l) mm long, included (vs. exserted from mouth of corolla) stamens with smooth (vs. papillose) filaments, and thecae 2.8–3.5 (vs. 5.2–6.5) mm long. *Aphelandra tridentata* is presently known only from central and northern Costa Rica. *Aphelandra kuna* can be distinguished from the wide-ranging *A. aurantiaca* by its eglandular (vs. glandular) pubescent bracts with 3–4 (vs. 5–15) marginal teeth per side, eglandular (vs. glandular) bracteoles that vary from 19 to 31 (vs. 7–15) mm in length, purplish (vs. orangish or red) corollas that vary from 43 to 45 (vs. 50–63) mm in length, included (vs. exserted from mouth of corolla) stamens, and thecae 2.8–3.5 (vs. 4–5) mm long. *Aphelandra kuna* might also be mistaken for *A. dolichantha* Donn. Sm. (cf. "dolicantha" in Durkee, 1978, and D'Arcy, 1987), an unusual species that also occurs near the crest of the El Llano–Cartí Road. *Aphelandra dolichantha* can be readily distinguished by its entire bracts subtending flowers with minute calyx lobes and white corollas. Further elucidation of relatives within the genus must await a detailed subgeneric treatment

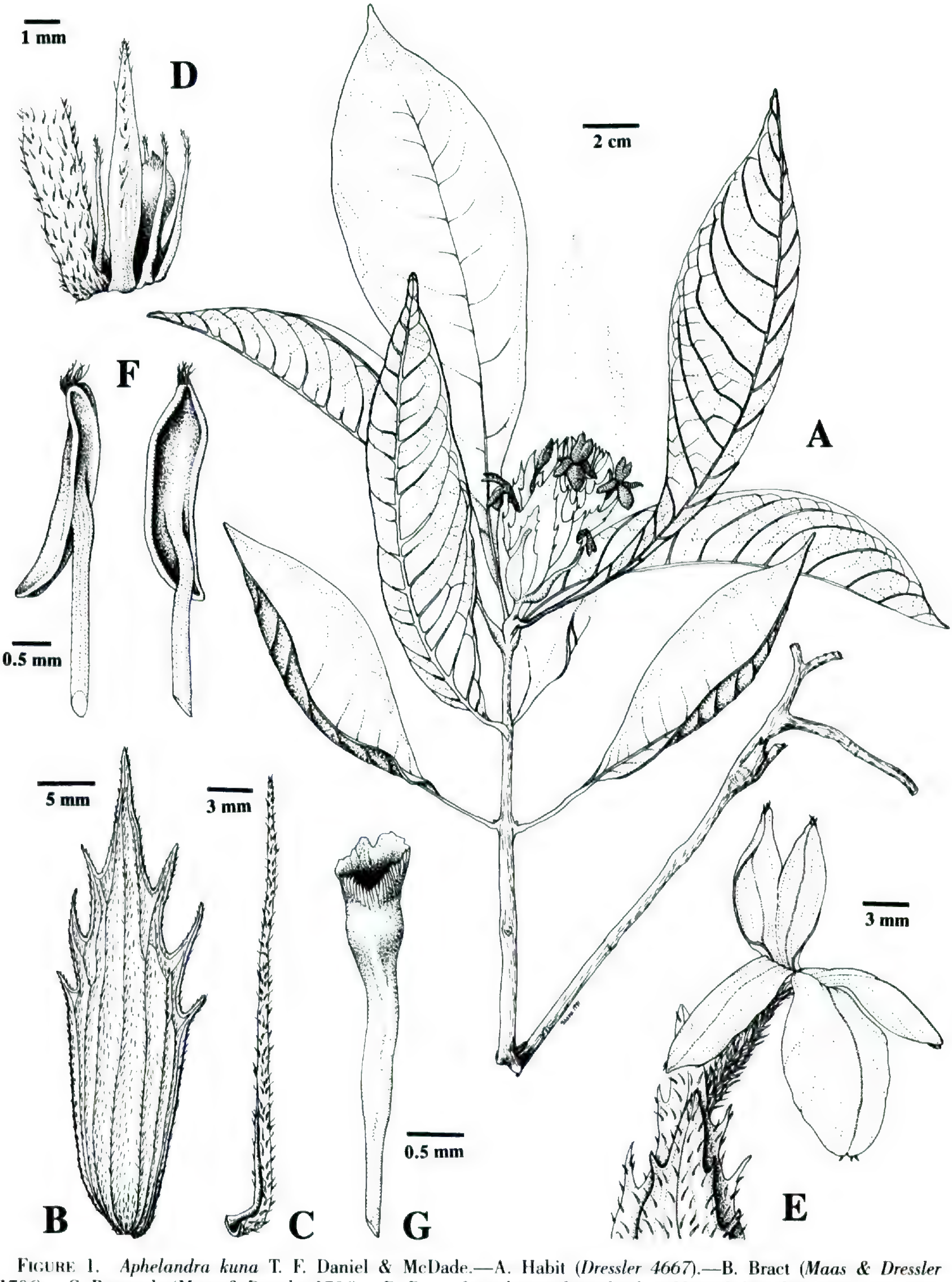

FIGURE 1. *Aphelandra kuna* T. F. Daniel & McDade.—A. Habit (*Dressler 4667*).—B. Bract (*Maas & Dressler 1706*).—C. Bracteole (*Maas & Dressler 1706*).—D. Base of one bracteole and calyx (*Maas & Dressler 1706*).—E. Apex of bract and corolla (*Dressler 4667*).—F. Anthers (*Dressler 4667*).—G. Distal portion of style (*Dressler 4667*). Drawn by Sheva Myers.

of *Aphelandra.* None of the South American species examined by us or treated by Wasshausen (1975) is likely to be confused with *A. kuna.* A revised key to all of the Panamanian species is provided below.

Pollen of *Aphelandra* is generally 3-colpate (Wasshausen, 1975; McDade, 1984; Daniel, 1991). Pollen of *A. kuna* (i.e., *Maas & Dressler 1706*) was observed with a scanning electron microscope. All pollen was either partially collapsed or otherwise degraded. Although the number and type of apertures from this collection could not be determined, the exine surface is reticulate as in some other species of the genus.

The specific epithet is derived from the Kuna people who live in the region where this species occurs and who are making concerted efforts to preserve native plant communities in the Serranía de San Blas.

Species Newly Reported from Panama

APHELANDRA SCOLNIKAE LEONARD

This species was described by Leonard (1953) from plants collected at 1700–2500 m elevation in several passes in the mountains surrounding Medellín, Antioquia Province, Colombia. Until recently, the species was known only from this region. A collection from Panama (Darién: Cerro Mali, ca. 22 km E of Pucuro, 1250–1500 m, 20–26 Oct. 1987, *G. de Nevers et al. 8475,* CAS, MO) extends the range of this unusual species considerably. *Aphelandra scolnikae* is a member of the *A. pulcherrima* (Jacq.) Kunth complex (McDade, 1984); within that group, it is a member of the lineage marked by patches of numerous small (less than 0.1 mm in diameter) extrafloral nectaries on the floral bracts. Plants of *A. scolnikae* can be distinguished from others in this lineage by their suffrutescent to treelet habit (vs. monocaulous herbs), relatively small leaves (up to about 22 cm long vs. up to 40 cm long in other species), and densely pubescent (vs. glabrous to puberulent) floral bracts with comparatively few extrafloral nectaries per glandular patch (mean of about 50 vs. more than 100 in other species).

The following key distinguishes all of the species of *Aphelandra* now known to occur in Panama:

1a. Floral bracts marginally toothed.
  2a. Floral bracts with paired lateral patches of nectariferous glands.
    3a. Floral bracts apically obtuse, 14–20 mm wide, usually entire, rarely with 2–3 pairs of minute marginal teeth each less than 1 mm long; corolla 65–71 mm long .... *A. sinclairiana* Nees
    3b. Floral bracts apically attenuate, 6–9 mm wide, with 2–4 pairs of well-developed marginal teeth each 1–2 mm long.
      4a. Corolla 36–41 mm long, thecae 3–4 mm long; plants of lowland deciduous forests and edges .... *A. scabra* (Vahl) Sm.
      4b. Corolla 55–70 mm long; thecae 4–5 mm long; plants of cloud forests .... *A. panamensis* McDade
  2b. Floral bracts lacking nectariferous glands.
    5a. Floral bracts less than 15 mm long; corollas less than 20 mm long.
      6a. Mature cauline leaves less than 10 cm long; floral bracts imbricate, ovate, with 2–3 well-developed teeth on each side of margin .... *A. seibertii* Leonard
      6b. Mature cauline leaves greater than 10 cm long; floral bracts lax, lanceolate, teeth minute (occasionally lacking).
        7a. Leaves glabrous to sparsely pubescent, apically acute; corolla red or pink .... *A. arnoldii* Mildbr.
        7b. Leaves hispid, with trichomes exceeding 1 mm long, usually slightly attenuate apically; corolla white to purplish .... *A. tonduzii* Leonard
    5b. Floral bracts greater than 20 mm long; corolla greater than 40 mm long.
      8a. Floral bracts pubescent with glandular trichomes, marginal teeth 5–15 per side; bracteoles glandular, 7–15 mm long; corolla orangish or red, 50–63 mm long; stamens exserted from mouth of corolla, thecae 4–5 mm long .... *A. aurantiaca* (Scheidw.) Lindl.
      8b. Floral bracts pubescent with eglandular trichomes, marginal teeth 3–4 per side; bracteoles eglandular, 19–31 mm long; corolla purplish, 43–45 mm long; stamens included in corolla tube, thecae 2.8–3.5 mm long .... *A. kuna* T. F. Daniel & McDade
1b. Floral bracts entire.
  9a. Floral bracts with paired lateral patches of nectariferous glands.
    10a. Nectariferous glands numerous (at least 50, usually more than 100) per patch and minute (each ca. 0.1 mm diam.).
      11a. Floral bracts more than 30 mm long, apically rounded and reflexed; calyx segments much shorter than floral bracts; capsules ca. 35 mm long .... *A. darienensis* Wassh.
      11b. Floral bracts less than 20 mm long, apically obtuse to acute to acuminate, not reflexed; calyx segments equaling or longer than floral bracts; capsules to 30 mm long.
        12a. Floral bracts distantly spaced along rachis, lax .... *A. laxa* Durkee
        12b. Floral bracts closely adjacent or imbricate, rachis not visible during anthesis.

13a. Floral bracts relatively narrow, width no more than ⅔ of length.
  14a. Plants rarely taller than 1.5 m; corolla 55–60 mm long ... *A. lingua-bovis* Leonard
  14b. Plants taller than 1.5 m when reproductive; corolla longer than 60 mm.
    15a. Spikes sessile, strongly tetrangular; floral bracts sparsely and minutely puberulous ... *A. leonardii* McDade
    15b. Spikes pedunculate, nearly terete in cross section; floral bracts conspicuously pubescent ... *A. scolnikae* Leonard
13b. Floral bracts relatively broad, width exceeding ¾ of length.
  16a. Bracteoles strongly falcate, 7–10 mm long, 3–5 mm wide; corolla usually yellow (rarely orange); capsules 28–31 mm long ... *A. hartwegiana* Nees
  16b. Bracteoles slightly falcate, 11–13 mm long, 2–3 mm wide; corolla orange; capsules 20–24 mm long ... *A. campanensis* Durkee

10b. Nectariferous glands 10 or fewer per patch, each > 0.2 mm diam.
  17a. Floral bracts green to occasionally dull brown-orange, narrowly ovate to ovate, to 7 mm wide.
    18a. Leaves glabrous; floral bracts 5–8 mm long, not imbricate, visible internodes 7–10 mm long ... *A. gracilis* Leonard
    18b. Leaves pubescent with trichomes densest on veins; floral bracts longer than 8 mm, slightly to closely imbricate, internodes not visible during anthesis.
      19a. Trichomes of distal stems erect; floral bracts slightly imbricate, apically obtuse; corolla pubescent with trichomes of tube exceeding 0.5 mm long ... *A. gracilis* × *sinclairiana*
      19b. Trichomes of distal stems appressed; floral bracts imbricate, apically acute; corolla minutely puberulent with trichomes of tube less than 0.2 mm long ... *A. golfodulcensis* McDade
  17b. Floral bracts orange, broadly ovate, 8–20 mm wide.
    20a. Distal stems, leaves, and bracts sparsely pubescent; floral bracts 10–12 mm long, 8–10 mm wide; corolla 57–62 mm long ... *A. terryae* Standl.
    20b. Distal stems, leaves, and bracts pilose; floral bracts 15–20 mm long, 14–20 mm wide; corolla 64–71 mm long ... *A. sinclairiana* Nees

9b. Floral bracts lacking nectariferous glands.
  21a. Stems and leaves somewhat fleshy; floral bracts longer than 30 mm; calyx reduced to minute, hyaline lobes ca. 1 mm long; corolla longer than 30 mm, white ... *A. dolichantha* Donn. Sm.
  21b. Stems and leaves not fleshy; floral bracts less than 10 mm long; calyx well developed, lobes ca. 5 mm long; corolla less than 20 mm long, red, pink, purplish, or white.
    22a. Leaves glabrous to sparsely pubescent, apically acute; corolla red or pink ... *A. arnoldii* Mildbr.
    22b. Leaves hispid with trichomes greater than 1 mm long, usually slightly attenuate apically; corolla white to purplish ... *A. tonduzii* Leonard

JUSTICIA EPHEMERA LEONARD

Justicia is both the largest genus of Acanthaceae and the most species-rich in Panama with 23 species (Daniel & Wasshausen, 1990). With more than 70 species of *Justicia* known from neighboring Colombia and given the still rudimentary knowledge of the eastern Panamanian flora, additional range extensions and new species are to be expected. A recent Panamanian collection of *J. ephemera* (Darién: Río Pirre, near El Real, 1–2 km upstream of water intake station for town of El Real, Feb. 1985, *L. McDade 687,* DUKE, MO, PMA) represents just such an extension. *Justicia ephemera* was previously known from northwestern Colombia (near Turbo in the department of Antioquia, Leonard, 1958). The Panamanian collection is not distinguishable from this species and shares with it a number of distinctive features: pandurate leaves with auriculate bases, bifariously pubescent stems with retrorse trichomes, densely flowered verticillate inflorescences, and short white corollas. This combination of character states is not shared by any other species of *Justicia* in Panama.

ODONTONEMA CUSPIDATUM (NEES) KUNTZE

Here and below, we bring the treatment of Panamanian *Odontonema* into accord with those of Baum (1982) and Daniel (1995), and we provide a key to the species. Durkee (1978) recognized five species of *Odontonema* in Panama. Three of these, *O. flagellum* (Oerst.) Kuntze, *O. longifolium* (Oerst.) Kuntze, and *O. strictum* (Nees) Kuntze, were included in the synonymy of *O. tubaeforme* (Bertol.) Kuntze by Baum (1982, as "tubiforme") and were similarly treated by D'Arcy (1987). *Odontonema callistachyum* (Schltdl. & Cham.) Kuntze, a species restricted in distribution to Mexico, Belize, and Guatemala, is neither known from Panama nor synonymous with *O. tubaeforme* (cf. D'Arcy, 1987). *Odontonema cuspidatum* has been reported from Mexico and the West Indies (Baum, 1982; Daniel, 1995) and is much cultivated throughout tropical regions. A collection from central Panama (Panamá: Cerro Azul, near Goofy Lake, 17 July 1962, *J. Dwyer 2076B,* MO, WIS) represents this species and the first report

of its occurrence in Panama. Collection notes indicate that the plant grew in a thicket. It is not known whether the occurrence of *O. cuspidatum* at this locality represents an indigenous population, naturalized plants, or a remnant from cultivation. The specimen was annotated by Durkee and cited in the *Flora of Panama* (Durkee, 1978) as *O. callistachyum.*

ODONTONEMA RUBRUM (VAHL) KUNTZE

Baum (1982) cited collections of *O. rubrum* from Panama, Colombia, and Venezuela. We have studied these and other specimens of *O. rubrum* from Panama and concur that this species is distinct from other Panamanian *Odontonema.* However, as noted by Baum (1982: 73), plants from the western portion of the range of *O. rubrum* (i.e., eastern Panama and northwestern Colombia) are rather different from plants from eastern Colombia and Venezuela. Our understanding of this species (and of other taxa with ranges including both Panama and Colombia) will benefit greatly from additional collecting in the poorly known areas of eastern Panama and adjacent Colombia.

Odontonema rubrum has been collected in the Canal Area, Colón, Darién, Panamá, and the Comarca de San Blas. Representative specimens of *O. rubrum* from Panama include:

Canal Area: drowned forest of Q. Ancha, 18 Dec. 1934, *C. Dodge & J. Steyermark 17043* (MO). **Colón:** Río Boquerón, near Peluca, 27 Jan. 1973, *R. Dressler 4263* (MO). **Darién:** between upper Río Membrillo and Camp 7 on road to San Blas, *J. Duke 10915* (MO, US). **Panamá:** 25 km NE of Cerro Azul on Río Pedras, 25 Nov. 1974, *S. Mori & J. Kallunki 3471* (MO). **Comarca de San Blas:** lower Río Ailigandi, 17 Jan. 1967, *J. Duke 9321* (MO).

Species of *Odontonema* in Panama can be distinguished by the following key:

1a. Plants often decumbent; mature cauline leaves less than 50 mm long .... *O. microphyllum* Durkee
1b. Plants erect; mature cauline leaves greater than 50 mm long.
  2a. Dichasia sessile, at least some (often most) whorled at inflorescence nodes; rachis pubescent with flexuose to retrorse to appressed trichomes 0.2–1 mm long, trichomes usually concentrated in 2 or more lines .... *O. tubaeforme* (Bertol.) Kuntze
  2b. Dichasia pedunculate, alternate or opposite at inflorescence nodes; rachis evenly pubescent with erect to flexuose to antrorse trichomes 0.05–0.3(–0.5) mm long.
    3a. Corolla externally pubescent with eglandular trichomes, tube abruptly expanded near the midpoint into a prominent throat, bent at point of expansion, upper lip 6–13 mm long; pollen 3-aperturate .... *O. rubrum* (Vahl) Kuntze
    3b. Corolla externally glabrous, tube barely or gradually expanded distally into a ± distinct throat, straight, upper lip 2–5 mm long; pollen 4-aperturate .... *O. cuspidatum* (Nees) Kuntze

SANCHEZIA LUTEA LEONARD

Leonard & Smith (1964) recognized 59 species of *Sanchezia.* Most of these are endemic to Peru. The only species previously reported from Panama is *S. pennellii* Leonard (Durkee, 1978; D'Arcy, 1987). Recent collections from eastern Panama have resulted in the addition of two other species of *Sanchezia* to the flora of Panama. A collection of *Sanchezia lutea* (Darién: Río Pirre, trail up river from house of Bartolo, 16 Mar. 1973, *H. Kennedy 2883,* TEX, US) was so annotated by Durkee in 1975, but neither the name nor the collection were subsequently listed by him (Durkee, 1978). Another collection from the same general region (Darién: along Río Pirre just above Chocó village of Pijibasal, 23 Feb. 1985, *L. McDade 804,* DUKE, MO) also represents this species. *Sanchezia lutea* was heretofore known only from various localities in Colombia (Leonard & Smith, 1964). The distinctions among the species of *Sanchezia* now known from Panama are discussed below.

SANCHEZIA PARVIBRACTEATA SPRAGUE & HUTCH.

This species was described from plants cultivated at the Royal Botanic Gardens at Kew without reference to its place of origin. It is widely cultivated in the tropics and under glass in temperate regions. Among the specimens cited by Leonard & Smith (1964), all appear to represent cultivated material except for a collection (*Cuatrecasas 11231,* US) from "banks of the Río Güamués" from the Intendencia de Putumayo in Colombia. A recent Panamanian collection of *S. parvibracteata* (Darién: Río Cocalito, 6 Feb. 1982, *C. Whitefoord & A. Eddy 60,* BM), presumably from an indigenous population, was also collected on a river bank.

The Panamanian species of *Sanchezia* can be distinguished by the following key:

1a. Bracts 30–60(–75) mm long, 20–50 mm wide, the pair at a node fused from base for up to 35 mm toward apex thereby forming cuplike involucres along the rachis; bracteoles and calyx concealed by bracts; corolla externally glabrous .... *S. pennellii*

1b. Bracts 6–20 mm long, 1.8–16 mm wide, free to base, not forming cuplike involucres; bracteoles and calyx surpassing bracts, clearly visible; corolla externally pubescent distally.
   2a. Bracts acute at apex; thecae 5–7 mm long .... *S. parvibracteata*
   2b. Bracts gradually acuminate at apex; thecae 3.5–4.5 mm long .... *S. lutea*

Cultivated Species

In treating the Acanthaceae of Panama, Durkee (1978) made an admirable attempt to include cultivated species as well as native and naturalized taxa. Numerous additional cultivated Acanthaceae are likely to be encountered in Panama beyond the two additions noted below.

Pachystachys lutea Nees

This species, a native of Peru, is widely cultivated for ornament in the American tropics and in temperate regions under glass. The shrubs are recognizable by their erect, quadrangular spikes of large (up to 25 mm long), bright yellow bracts subtending white flowers. In Panama, plants occur in cultivation outdoors (Canal Zone: western edge of Panama City near Smithsonian Tropical Research Institute, 22 Feb. 1988, *T. Daniel et al. 5455*, CAS). There are no reports of these plants becoming naturalized in Panama.

Justicia betonica L.

This species is a native of the Indian subcontinent and eastern tropical Africa. *Justicia betonica* is a shrub distinguishable from all other species of *Justicia* in Panama by its long (up to 16 cm), lax spikes with white to pinkish (with dark purple markings) corollas subtended by relatively large (ca. 10 mm long) bracts that are white with dark green venation. It is sometimes cultivated as a hedge or garden plant in warm tropical regions, and although not listed in *Hortus Third* (Bailey Hortorium, 1976), is cultivated in sheltered locations in Florida and under glass elsewhere in the United States. In Panama, plants are cultivated in yards (Coclé: La Mesa along road between El Valle de Anton and Cerro Gaital, 24 Feb. 1988, *F. Almeda et al. 5927*, CAS). It is known to become naturalized in some tropical regions (e.g., Hawai'i) and may do so in Panama.

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REVISIÓN TAXONÓMICA DEL GÉNERO *ALEXA* MOQ. (FABACEAE, SOPHOREAE)[1]

Nelson Ramírez[2]

Resumen

Este trabajo reconoce nueve especies del género *Alexa*: *A. bauhiniiflora*, *A. canaracunensis*, *A. confusa*, *A. cowanii*, *A. grandiflora*, *A. herminiana*, *A. imperatricis*, *A. surinamensis* y *A. wachenheimii*. Los caracteres morfológicos reproductivos representan la base para la delimitación de los taxa, específicamente el tipo de inflorescencia, longitud de las flores, forma y pubescencia de los pétalos, numero y dimensión de los lóbulos del cáliz y longitud del gineceo (estípite, ovario y pistilo). El color de los folíolos aporta valor diagnóstico entre algunas especies. La anatomía del mesófilo de la hoja examinada en las especies venezolanas representa otro carácter importante, aunque existe variación y superposición. En base a los estudios morfoanatómicos se realizaron ilustraciones y claves para las especies. Además, se recopiló información sobre la distribución geográfica, habitat, biología de reproducción, nombres comunes y la importancia económica de las especies.

Abstract

Nine species of *Alexa* are recognized: *A. bauhiniiflora*, *A. canaracunensis*, *A. confusa*, *A. cowanii*, *A. grandiflora*, *A. herminiana*, *A. imperatricis*, *A. surinamensis*, and *A. wachenheimii*. Based on vegetative and reproductive characters, this paper provides descriptions, illustrations, and a key to the species of *Alexa*. The species limits were determined mainly by reproductive characters: inflorescence type, flower length, petal form and vestiture, number and size of calyx lobes, and gynoecium size (gynophore, ovary, and pistil). Leaflet form and color are diagnostic for some species. The anatomical study of the Venezuelan species of *Alexa* represents an important character in species delimitation, although there is some overlap. Additional information is provided concerning geographic distribution, habitat, reproductive biology, common names, and economic uses.

Alexa Moq. (Fabaceae) es un género arbóreo de distribución exclusivamente neotropical, el cual pertenece a la tribu Sophoreae L. La revisión más reciente realizada por Yakovlev (1977) fue publicada en ruso utilizando principalmente caracteres vegetativos, y no examinó los especímenes depositados en el Herbario Nacional de Venezuela (VEN), siendo en Venezuela donde ocurren la mayoría de las especies. El objetivo de este trabajo es realizar una revisión taxonómica del género *Alexa*, utilizando el mayor número de carácteres reproductivos y vegetativos. Además, se evalúa la anatomía foliar para las especies venezolanas.

Historia del Género

En 1845, Robert Schomburgk describió una especie bajo el nombre genérico de *Alexandra*, el cual resultó homónimo de un género de Chenopodiaceae. Esta situación indujo a Moquin-Tandon (1849) a crear el nombre de *Alexa* para el género originalmente descrito por Schomburgk. La primera revisión del género no se realizó hasta que Yakovlev (1977) reseñó nueve especies, dos de las cuales eran nuevas para la ciencia. Más recientemente Ramírez & Agostini (1987) señalan cinco especies para Venezuela.

Posición Sistemática y Relaciones Intergenéricas

El género *Alexa* ha sido ubicado en la tribu Sophoreae, desde mediados del siglo pasado (Bentham & Hooker, 1865; Pittier, 1944; Hutchinson, 1964; Polhill, 1981). Sin embargo, Yakovlev (1972) dividió la tribu Sophoreae en tres tribus: Baphieae,

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Acosmieae y Angylocalyceae, ubicando a *Alexa* en la tribu Angylocalyceae. Aún cuando la tribu Sophoreae se ha incluida tradicionalmente en las Fabaceae, su posición sistemática ha permanecido controversial. Básicamente, Papilionoideae se distingue de Caesalpinioideae por la prefloración imbricada de los pétalos y por el vexilo externo. Sin embargo, algunos géneros de Fabaceae en la tribu Sophoreae presentan una ligera e inestable imbricación de los pétalos o han perdido el pétalo vexilar (Peltier, 1972; Yakovlev, 1972). Por esta razón, la tribu Sophoreae es considerada por Polhill (1981) como un taxon heterogéneo de conveniencia entre Caesalpinioideae y Papilionoideae con una gran diversidad en flores, frutos y semillas.

Polhill (1981) reorganiza a los 48 géneros de la tribu Sophoreae en "grupos," los cuales incluyen a los taxa más relacionados y le asigna el nombre de uno de los géneros incluidos. De acuerdo a este sistema *Alexa* pertenece al "grupo" Dussia, el cual se caracteriza por presentar hojas imparipinadas, cáliz valvado a ligeramente imbricada, lóbulos subiguales o el par superior frecuentemente más ancho, algunas veces abierto irregularmente; hipanto bien desarrollado; pétalos carinales libres o adnatos en el lado más bajo y estambres libres, cortamente o irregularmente unidos en la base. En este contexto, *Alexa* se encuentra más estrechamente relacionada con *Dussia, Petaladenium, Monopteryx, Spirotropis, Uleanthus, Panurea, Clathrotropis, Diplotropis* y *Bowdichia*. *Alexa* se diferencia de todos estos géneros por el cáliz más o menos cerrado en la yema, con lóbulos cortos; los pétalos grandes, naranjados o rojos, de los cuales cuatro son similares; bracteólas pequeñas y gruesas en la base del pedicelo; fruto leñoso y dehiscente; y semillas más o menos discoidales (Polhill, 1981). Por otra parte, Lewis & Owen (1989) destacan las observaciones de C. H. Stirton, quien reseña la estrecha afinidad del género *Alexa* con el genero monotípico australiano *Castanospermum*. Esta observación taxonómica es reforzada por la presencia de un alcaloide (castanospermina) en ambos géneros (Nash et al., 1988).

Polinización

Alexa posee flores grandes y llamativas; el hipanto y cáliz forman una estructura que produce y almacena el néctar. Las flores presentan diferentes colores en algunos casos contrastante. Polhill (1981) reportó que las flores de *Alexa* son polinizadas por aves, y van der Pijl (1941) señaló que la polinización en *Alexa* es realizada por aves o murciélagos. Butanda-Cervera et al. (1978) encontraron que *Glosophaqa soricina* (Phyllostomatidae) lleva polen de *A. grandiflora* Ducke como parte del contenido estomacal. Observaciones en *A. canaracunensis* Pittier mostraron que posee una antesis nocturna y es polinizada por una especie de murciélago del género *Choeroniscus* (Phyllostomatidae) (Sobrevila & Linares, com. pers.). La variación encontrada en el modo de polinización puede estar asociada con la gran variación que existe en la morfología de los granos de polen reportada por Ferguson & Skvarla (1981).

Dispersión

No existe información publicada sobre la dispersión de semillas en el genero *Alexa*, pero campesinos de la zona de Río Grande Estación del Ministerio del Ambiente (MARNR) de El Palmar, Estado Bolívar, señalan que las semillas de *A. imperatricis* (Schomb.) Baillon son removidas del suelo por lapas (*Cuniculus paca*) y picures (*Dasyprocta* sp.).

Importancia Económica

La madera de *A. imperatricis* es utilizada para tablones y puentes provisionales. Van der Slooten (1970) y Paula (1977) reportan la utilidad de la madera de *A. imperatricis* para la construcción de interiores, contraenchapado y manufactura de papel. Además, la corteza es usada como poción para matar peces. Estudios recientes han demostrado la presencia de una sustancia denominada castanospermina en ocho especies del genero *Alexa* (Nash et al., 1988), la cual exhibe actividad in vitro contra el virus del sida (Tyms et al., 1987).

Materiales y Métodos

El material de herbario para el estudio sistemático del género *Alexa* consistió en las colecciones depositadas en el Herbario Nacional de Venezuela (VEN), Herbario de la Facultad de Agronomia (MY), Missouri Botanical Garden (MO), del New York Botanical Garden (NY) y del Smithsonian Institution (US). Además, se realizaron nuevas colecciones en los Estados Bolívar y Delta Amacuro por el autor. Las descripciones de las especies fueron realizadas en un patrón similar, sin embargo, y debido a la carencia de colecciones, las descripciones de algunas de las especies están basadas solo en aquellos caracteres que se pudieron evaluar.

Estudio anatómico

Los folíolos de las especies venezolanas del género *Alexa* fueron estudiados de material de her-

bario. Las muestras fueron colocadas en alcohol al 50% por 24 horas, luego transferidas a alcohol al 70% para restablecer la forma de las células del tejido a estudiar. Los cortes histológicos de los folíolos fueron realizados en la parte media de folíolos centrales. Previa decoloración, los cortes de los folíolos fueron coloreados con azul de toluidina y preparados en forma semipermanente. En cada muestra examinado se caracterizaron los diferentes tejidos y las formas de las células constituyentes. Las dimensiones de las mismas fueron realizadas con un micrómetro. Las epidermis fueron tratadas utilizando el método de aclaramiento en cloro. Este método consiste en hervir los folíolos en una solución de cloro comercial, hasta que ocurra el aclaramiento total del tejido; después fueron coloreados y montados en preparaciones semipermanentes.

Tratamiento Taxonómico

Alexa Moq. in DC., Prodr. 13(2): 168. 1849. *Alexandra* R. H. Schomb., Lond. J. Bot. 4: 12. 1845 (non *Alexandra* Bunge, Linnaea 12: 120. 1843). TIPO: *Alexa imperatricis* (Schomb.) Baillon, Hist. Pl. 2: 362. 1870.

Arbol 10–35 m alto; yemas concaulescentes. Hojas imparipinnadas, estípulas y estipelas ausentes, pecíolo terete, glabro o pubescente; raquis terete, semiterete o comprimido y/o aquillado, glabro o pubescente; peciólulo terete o semiterete, rugoso, glabro o pubescente; folíolos (5–)7–11(–17), generalmente opuestos, membranáceos o subcoriaceos, nervios poco prominentes, de forma oblonga, ovada, obovada o elíptica, base obtusa u ocasionalmente asimétrica, ápice abruptamente o angostamente largo o corto acuminado, pubescencia abaxial o ausente, ocasionalmente en la cara adaxial, color verde marrón amarillento. Inflorescencias terminales u ocasionalmente en tallos viejos (caulifloras), generalmente racimos o panículas racemiformes, con 6–80(–160) flores por inflorescencia; pedúnculo de la inflorescencia densamente pubescente, con pelos rojizos o marrones oscuros, ocasionalmente lenticelada; eje densamente pubescente, ocasionalmente pelos dispersos; brácteas ovadas lanceoladas y acuminadas; bracteólas pediceladas o sésiles, ovadas, lanceoladas y acuminadas. Flores hermafroditas; pedicelo floral terete, pubescente; hipanto acampanado, semiacampanado, incurvo o recto, coriáceo o subcoriáceo, pubescencia externa densa y marrón oscura, ocasionalmente glabro; cáliz acampanado, semiacampanado, incurvo o recto, coriáceo o subcoriáceo, pubescencia densa externamente y glabra internamente o con pelos esparcidos sobre los lóbulos, casi trunco, 1(–2) profundamente partido 3–5 lobado, con pequeños apículos; vexilo elíptico, membranáceo o subcoriáceo, glabro o pubescente, semielíptico u obovado, base trunca, ápice emarginado, bordes lisos u ondulados; alas obovadas, elípticas o lanceoladas, membranáceas o sub-coriáceas; glabras o pubescentes, base trunca, ápice de agudo a obtuso; bordes lisos u ondulados; pétalos carinales lanceolados u obovados, generalmente falcados, membranáceas o subcoriaceos, glabros o pubescentes, base trunca, ápice de agudo a obtuso, acuminado o emarginado, bordes lisos u ondulados; androceo formado por 10–12 estambres libres, rara vez 7–10 estaminodios; anteras dorsifijas, versátiles, con dehiscencia longitudinal; ovario de elipsoide a fusiforme, ligeramente comprimido, pubescente, con 4–9 óvulos; ginóforo glabro o pubescente; estilo glabro, recto o incurvo; estigma inconspicuo. Frutos dehiscentes, leñosos, cáliz persistente en el fruto, ápice generalmente agudo, pubescencia densa y marrón o marrón rojiza, glabrescentes, con 1–5(–7) semillas. Semillas ovoides o elipsoides, glabras; cubierta seminal lisa, marrón oscuro; embrión recto o ligeramente curvo; radícula y plúmula poco desarrolladas o inconspicuas; endosperma ausente.

Clave Analítica Para las Especies del Género *Alexa*

1. Cáliz trunco o 1(–2) lobulado, con o sin 5–6 apículos pequeños; pétalos de membranáceos a subcoriáceos; folíolos con ápice abruptamente largo o corto acuminado 2

1. Cáliz conspicuamente 3–5 lobulado, con o sin apículos pequeños en los ápices de los lóbulos; pétalos de subcoriáceos a coriáceos; folíolos con ápice de acuminado a angostamente acuminado 5

2(1). Ovario 1.5–3.9 cm largo, el ginóforo 1.5–5.1 cm largo; cáliz con lóbulos 0.5–1.9 cm largo; apículos del cáliz 1–4 mm largo; pétalos glabros; pedicelo floral 2.0–6.5 cm largo; bracteólas 0.4–0.8 mm ancho; folíolos de membranáceos a subcoriáceos 3

2. Ovario 0.9–1.4 cm largo, el ginóforo 0.25–1.2 cm largo; cáliz con lóbulos 0.2–0.5 cm largo; apículos del cáliz 0.1–0.2 mm largo; pétalos pubescentes; pedicelo floral 0.2–1 cm largo; bracteólas 1–2 mm ancho; folíolos membranáceos 4

3(2). Flores 8.5–12.3 cm largo; cáliz densamente pubescente con lóbulos 1.3–1.9 cm largo; gineceo 7.1–12.2 cm largo; inflorescencia 30–35.5 cm largo; brácteas 0.9–1.2 cm largo; pedicelo floral 2–3.4 cm largo; folíolos densamente pubescentes en la cara abaxial ..... 1. *A. bauhiniiflora*

3. Flores 5–6 cm largo; cáliz glabro con lóbulos 0.5–1.1 cm largo; gineceo 4.8–5 cm largo; inflorescencia 10–21 cm largo; brácteas 0.2–0.3(–0.8) cm largo; pedicelo floral 4.2–6.5 cm

largo; folíolos glabros o pelos escasos sobre los nervios más prominentes en la cara abaxial .... 6. *A. herminiana*

4(2). Inflorescencia panícula racemiforme con tres flores en cada nudo, 40–55 flores por inflorescencia; cáliz 1–1.1 cm largo; gineceo 3–3.4 cm largo; estilo 1.5–1.6 cm largo, uncinado en el ápice; ginóforo pubescente, 0.25–0.3 cm largo; flores 4.5–5.1 cm largo; folíolos con pubescencia dispersa; los pelos cortos y adpresos .... 4. *A. cowanii*

4. Inflorescencia un racimo simple, 6–11 flores por inflorescencia; cáliz 1.5–2 cm largo; gineceo 8.3–9.4 cm largo; estilo 6.2–7 cm largo, curvado en la parte media; ginóforo glabro, 1–1.2 cm largo; flores 8–8.5 cm largo; folíolos con pubescencia abundante ..... 2. *A. canaracunensis*

5(1). Inflorescencia racimo simple o bifurcado terminal; pedúnculo de la inflorescencia (1–)3–30 cm largo; folíolos de membranáceos a subcoriáceos, glabros o pubescentes 6

5. Inflorescencia racimo simple caulifloro; pedúnculo de la inflorescencia 0.5–3.3 cm largo; folíolos subcoriáceos, glabros o pubescencia dispersa sobre los nervios más prominentes 8

6(5). Hoja 71–99 cm largo, folíolos 11–17, nervios prominentes en la cara abaxial, pubescentes por ambas caras; raquis 41–52 cm largo; inflorescencia 31–69(–158) cm largo; flores 6–9.5 cm largo; pedicelo floral 0.4–1.4 cm largo; bracteólas 0.5–1.5 cm ancho; estilo 2.1–3 cm largo .... 3. *A. confusa*

6. Hoja (21–)27–58 cm largo, folíolos 5–9, nervios poco prominentes en la cara abaxial, glabros o glabrescentes; raquis 10–27 cm largo; inflorescencia 5–21 cm largo; flores 3–5.6 cm largo; pedicelo floral 7–4.5 cm largo; bracteólas 0.2–0.5 cm largo; estilo 0.6–1.2 cm largo 7

7(6). Pecíolo 4–8 cm largo; bracteólas 0.2–0.4 cm ancho; cáliz 1.2–1.4 cm largo; alas lanceoladas 1–1.1 cm ancho; quilla lanceolada ca. 1 cm ancho; ovario 1.2–2.8 cm largo .. 5. *A. grandiflora*

7. Pecíolo 8–11 cm largo; bracteólas ca. 3–5 × ca. 5 mm ancho; cáliz 1.8–2.5 cm largo; alas obovadas, ca. 0.8 cm ancho; quilla oblonga 0.6–0.8 cm ancho; ovario ca. 1 cm largo .... 9. *A. wachenheimii*

8(5). Folíolos membranáceos, de elípticos a ampliamente elípticos, ápice largo o corto acuminado; semillas ca. 2.5 cm largo y ca. 2.3 cm ancho .... 8. *A. surinamensis*

8. Folíolos subcoriáceos, elípticos, ápice corto acuminado; semillas 3–3.2 cm largo y 2.3–3.3 cm ancho .... 7. *A. imperatricis*

1. Alexa bauhiniiflora Ducke, Bull. Mus. Hist. Nat. (Paris), sér. 2(4): 732. 1932. TIPO: *Ducke RB 5308* (holótipo, P no visto). Figura 1.

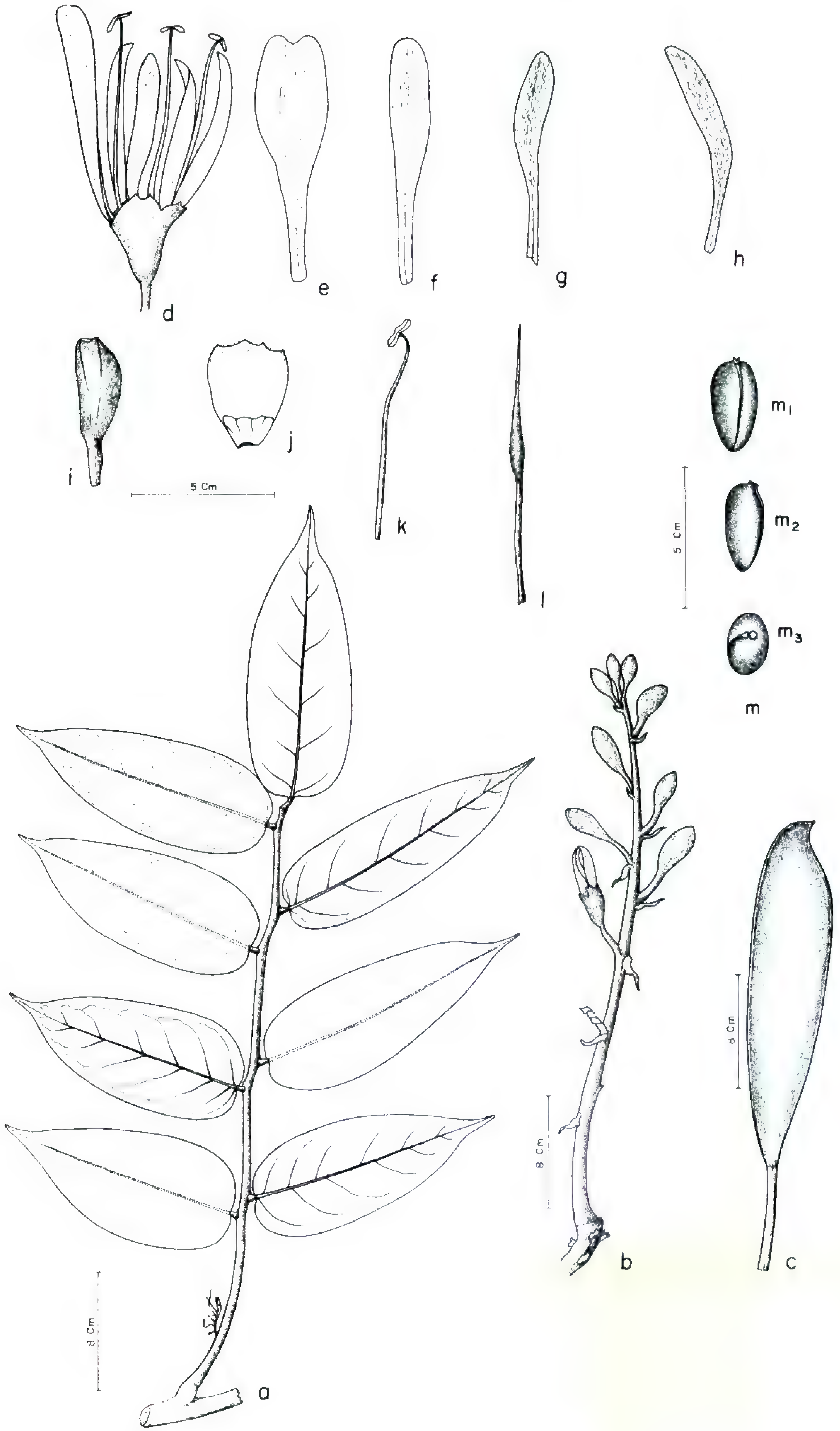
Arbol 6–10 m alto. Hojas 45–60 cm largo; pecíolo 7–11 cm largo, con pubescencia dispersa amarillenta; raquis terete o comprimido, 19–28 cm largo, con pubescencia dispersa amarillenta; peciólulo 0.6–1.1 cm largo, densamente pubescente; folíolos 7–9, de alternos a subopuestos, subcoriáceos, con nervios poco sobresalientes, ovados, color amarillento, 11–22 × (4–)6–9 cm, de pubescentes a glabrescentes abaxialmente, base obtusa, ápice angostamente corto acuminado. Inflorescencia racemosa, terminal, 30–35.5 cm largo, con 13–18 flores, eje pubescente de color amarillo verdoso, pedúnculo de la inflorescencia 11.3–14 cm largo; brácteas ovadas, acuminadas, 0.9–1.2 × 0.25–0.33 cm; bracteólas ovadas, acuminadas, 1.5–1.7 × 0.3–0.4 cm. Flores 8.5–12.3 cm largo; pedicelo floral terete y aquillado, 2–3.4 cm largo; hipanto acampanado, subcoriáceo, 0.6–0.9 cm largo, con pubescencia externa densa y de color marrón verdoso; cáliz acampanado, subcoriáceo, 1.7–2.4 cm largo, pubescencia externa densa e internamente glabra con pelos hacia el borde, lóbulo único 1.3–1.9 cm largo o con 5 apículos sobre el borde del cáliz 0.2–0.4 cm largo; vexilo obovado, membranáceo, 6.4–8.5 × 2.1–2.3 cm, pubescencia externa densa, ápice emarginado, borde liso; alas de lanceoladas a obovadas, falcadas, membranáceas, 5.6–7.6 × 0.5–0.75 cm, pubescencia externa, ápice agudo semiobtuso, bordes lisos; pétalos carinales lanceolados a obovados, falcados, membranáceos, 5.7–7.5 × 0.4–0.6 cm, pubescencia densa, ápice agudo a semiobtuso, bordes lisos; androceo formado por 8–10 estambres; filamentos 3.7–7.6 cm largo; anteras 1.1–1.2 × 0.2–0.25 cm; gineceo 7.1–12.2 cm largo; ovario con pubescencia amarillenta, 1.9–3.9 × 0.2–0.3 cm; ginóforo pubescente, 2.7–5.1 cm largo; estilo glabro y ligeramente recto, 3.1–3.2 cm largo. Fruto 20–23.8 × 4.5–4.9 cm, pubescencia marrón rojiza, con 2–3 semillas. Semillas 3–3.3 × 1.9–2.1 cm.

Distribución. En Venezuela al extremo sur del Amazonas venezolano y noroeste de Brasil. De acuerdo al mapa de vegetación de Venezuela (Huber & Alarcón, 1988), *Alexa bauhiniiflora* se distribuye en bosques siempreverdes.

Alexa bauhiniiflora se diferencia de las otras especies por presentar flores con lóbulos del cáliz y gineceo (ginóforo y ovario) más largos que sus congeneres.

→

FIGURA 1. *Alexa bauhiniiflora* Ducke.—a. Hoja.—b. Inflorescencia.—c. Fruto.—d. Flor.—e. Pétalo vexilar.—f. Alas.—g, h. Pétalos carinales.—i. Cáliz.—j. Cáliz desplegado, lado interno.—k. Estambre.—l. Pistilo.—m. Semilla: m_1 vista frontal, m_2 vista lateral, m_3 vista superior.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Especímenes examinados. BRASIL. **Amazonas:** Río Negro–Río Padauri, *Fróes 22528* (US). VENEZUELA. **Amazonas:** Sierra Parima, cabeceras de Río Siapa y Río Padauri, *Cardona 1473* (US).

2. Alexa canaracunensis Pittier, Bol. Soc. Venez. Ci. Nat. 7(51): 309. 1942. TIPO: *Cardona 420* (holótipo, VEN). Figura 2.

Arbol 10–30 m alto. Hojas 47–75 cm largo; pecíolo 12–23 cm largo, pubescencia densa y erecta; raquis de terete a semiterete 17–36 cm largo, pubescencia dispersa con mezcla de pelos erectos largos y cortos; peciólulo 0.5–0.8 cm largo, pubescencia densa o dispersa y erecta; folíolos 9–15, subopuestos a alternos, membranáceos, con nervios poco prominentes, oblongos-obovados o elípticos, de color verde claro, (8–)12–17(–13) × 4–7 cm, pubescencia densa abaxialmente, base obtusa, ápice abruptamente largo acuminado. Inflorescencia racimo terminal 15–17 cm largo, con 6–11 flores; eje con pelos largos de color marrón oscuro; pedúnculo de la inflorescencia 4.7–8.3 cm largo; brácteas obovadas a lanceoladas 0.8–1.7 × 0.2–0.3 cm; bracteólas obovadas triangulares, 1.5–3.0 × 1–1.5 mm. Flores 8–8.5 cm largo; pedicelo 2–3 mm largo; hipanto acampanado, subcoriáceo 0.9–1 cm largo, con pubescencia externa, densa de color marrón oscuro; cáliz acampanado, subcoriáceo, 1.5–2 cm largo, pubescencia externa, densa e internamente glabro con pelos esparcidos sobre los lóbulos, lóbulos 1(–2), 0.3–0.5 cm largo y con 5 apículos en el borde del cáliz 0.1–0.2 cm largo; vexilo de elíptico a semielíptico, membranáceo, 7.1–9.1 × 1.5–2.7 cm, glabro o glabrescente externamente, ápice emarginado, borde liso; alas lanceoladas y falcadas, membranáceas, 6.2–8.7 × 0.6–1 cm, glabras, ápice agudo a obtuso, bordes lisos a levemente ondulados; pétalos carinales lanceolados y falcados, membranáceos, 6–8.6 × 0.7–0.9 cm, glabros, ápice obtuso a levemente acuminado, borde liso a levemente ondulado; androceo formado por 10 estambres; filamentos 5.7–7.4 cm largo; anteras 1.0–1.3 × 0.1–0.2 cm; gineceo 8.3–9.4 cm largo; ovario con pubescencia marrón a dorada, 1–1.1 × 0.1–0.2 cm; ginóforo glabro, 1–1.2 cm largo; estilo glabro, incurvo en la parte media, 6.2–7 cm largo. Fruto 14–19.8 × 3.3–4.1 cm, pubescencia marrón rojiza, con 1–4 semillas. Semillas 2.6–3.4 × 1.4–1.6 cm.

Nombres comunes. Karapa, Kadapa, Kaiapa, Kayapa, Caicareño, Tunadi.

Distribución. En Venezuela al sur, sudeste y sudoeste del Estado Bolívar y en Brasil en el Estado Roraima. De acuerdo al mapa de vegetación de Venezuela (Huber & Alarcón, 1988), *Alexa canaracunensis* se distribuye en bosques siempreverdes, ombrófilos basimontanos siempreverdes.

Alexa canaracunensis se diferencia de las otras especies por presentar estilo incurvo y pedicelo floral incurvo en la parte media. Esta especie está más relacionada con *A. cowanii* pero difiere en inflorescencias tipo racimos y flores más largas.

Especímenes examinados. BRASIL. **Roraima:** Municipio de Boa Vista, Reserva Ecológica Maracá, *Lewis & Milliken 1683* (NY). VENEZUELA. **Bolívar:** Sabanas de El Canaracuni, cabecera del Río Caura, *Cardona 420* (VEN); Municipio Foráneo Aripao, Alto Río Caura, *Aymard 6698* (NY, PORT); El Dorado, *Bernardi 6941* (US).

3. Alexa confusa Pittier, Bol. Soc. Venez. Ci. Nat. 8(56): 262. 1943. TIPO: *Williams 11402* (holótipo, US). Figura 3.

Alexa superba Cowan, Mem. New York Bot. Gard. 8(2): 117. 1953. TIPO: *Cowan & Wurdack 31531* (holótipo, NY).

Arbol 10–30 m alto. Hojas 71–99 cm largo; pecíolo acanalado 9–17 cm largo con pubescencia densa y marrón amarillenta; raquis terete cuadrangular o comprimido 41–52 cm largo, con pubescencia rojiza de pelos largos y cortos; peciólulo 0.8–1.8 cm largo, con pubescencia densa de pelos blancos y rojizos, largos y cortos; folíolos 11–13(–17), de alternos a subopuestos, subcoriáceos, con nervios prominentes abaxialmente, oblongos, ovados u obovados, de color pardo, (7–)12–26 × 5–10 cm, pubescencia amarillenta adaxialmente, escasa sobre los nervios y muy densa abaxialmente, base obtusa simétrica o asimétrica, ápice abruptamente o angostamente corto o largo acuminado. Inflorescencia racemosa, terminal, 31–69(–158) cm largo, con 24–80(–160) flores; eje densamente pubescente de color marrón rojizo y con numerosas o escasas lenticelas; pedicelo de la inflorescencia 10–30(–35) cm largo; brácteas ovadas y acuminadas 5–9 × 1.5–3 mm; bracteólas ovadas, acuminadas, 3–5 × 0.5–1.5 mm. Flores 6–9.5 cm largo; pedicelo 0.4–1.4 cm largo, pubescente; hipanto acampanado e incurvo en la base, coriáceo, 0.6–1.8 cm largo, con pubescencia externa, densa y de color marrón oscuro; cáliz acampanado e incurvo, coriáceo, 2.2–2.6 cm largo, pubescencia externa, densa y de color marrón oscuro, internamente glabro con pelos dispersos sobre los lóbulos; lóbulos 3–5 grandes o 2 grandes y 3 pequeños apículos 0.2–0.7 cm largo, con los ápices agudos u obtusos; vexilo obovado y asimétrico, subcoriáceo cuando seco, 6.2–7.2 × 1.2–1.3 cm, pubescencia externa, ápice de levemente emarginado a emarginado, con bordes lisos; alas obovadas, subcoriáceas, 6.5–7.2

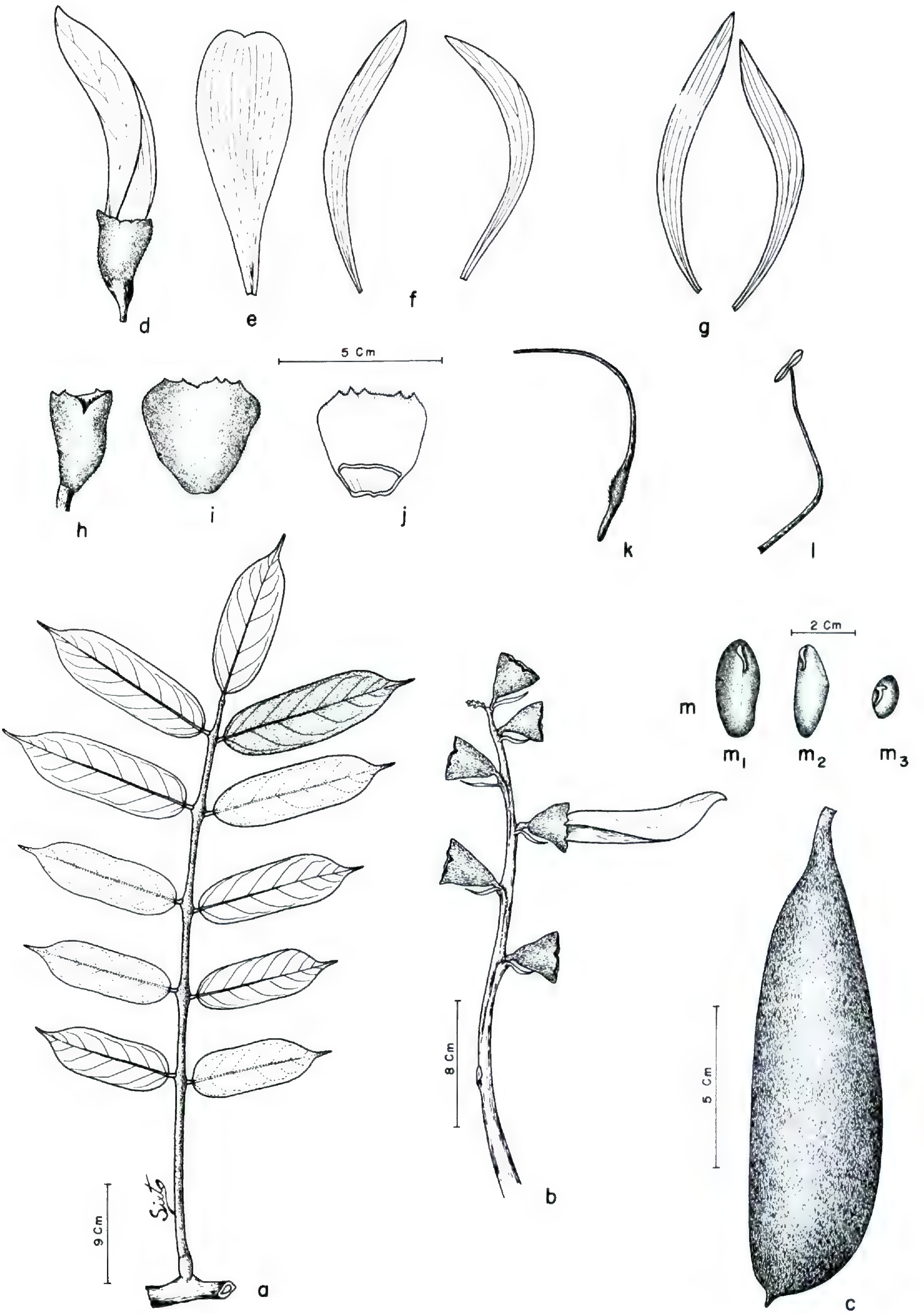
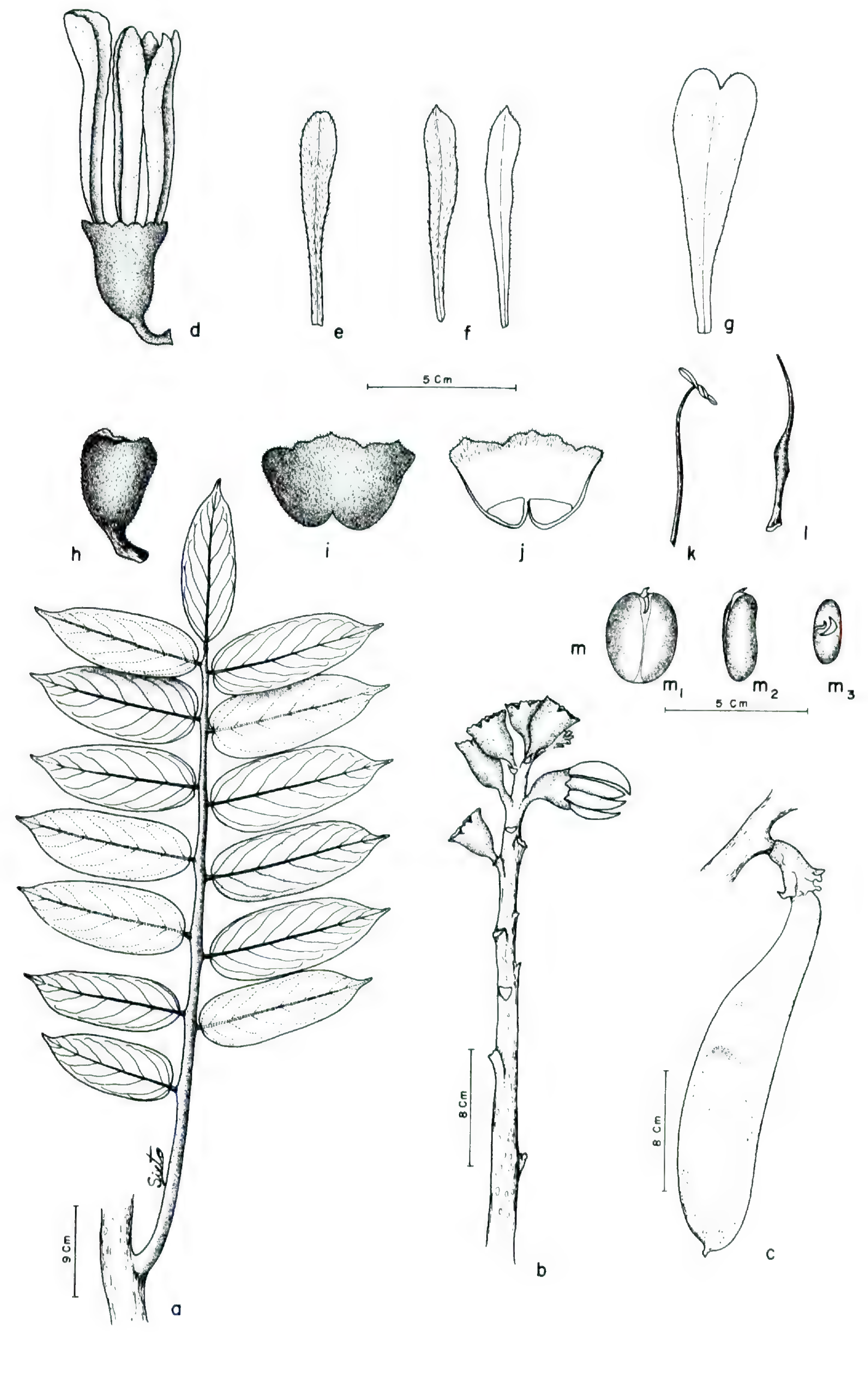


FIGURA 2. *Alexa canaracunensis* Pittier.—a. Hoja.—b. Inflorescencia.—c. Fruto.—d. Flor.—e. Pétalo vexilar.—f. Alas.—g. Pétalos carinales—h. Cáliz.—i. Cáliz desplegado, lado externo.—j. Cáliz desplegado, lado interno.—k. Pistilo.—l. Estambre.—m. Semilla: m_1 vista frontal, m_2 vista lateral, m_3 vista superior.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× 1–1.1 cm, pubescencia externa, ápice de levemente acuminado a obtuso, bordes lisos o levemente ondulados: pétalos carinales obovados, subcoriáceos, 4.5–8.9 × 2.2–3.3 cm; 1–2, cuando 1 el otro reducido o caedizo, pubescencia externa, ápice levemente emarginado, bordes de lisos a levemente ondulados; androceo formado de 10–15 estambres; filamentos 5.8–6.4 cm largo; anteras 0.9–1.7 × 0.08–0.2 cm; estaminodios 7–10 o ausentes; gineceo 6–6.2 cm largo; ovario densamente pubescente 1.3–1.7 × 0.4–0.45 cm; ginóforo pubescente, 1.4–1.5 cm largo; estilo glabro, ligeramente curvo, 2.1–3 cm largo. Fruto 20–26 × 4–5.5 cm, marrón rojizo, con 4–7 semillas. Semillas 2.9–3.3 × 3.1–3.4 cm.

Nombres comunes. Guamo peludo, Kure, Cule Yek (Arekuna).

Distribución. En Venezuela al sur, sudeste y sudoeste del Estado Bolívar y al centro y nordeste del Estado Amazonas, y en Brasil en el Estado Roraima. De acuerdo al mapa de vegetación de Venezuela (Huber & Alarcón, 1988), *Alexa confusa* se distribuye principalmente en bosques siempreverdes, y menos frecuentemente en bosques semideciduos, específicamente en bosques ombrófilos submontanos subsiempreverdes.

Alexa confusa se diferencia de las otras especies por presentar folíolos pubescentes en ambas caras, flores con 1 o 2 pétalos carinales, androceo ocasionalmente con 7 a 10 estaminodios.

Especímenes examinados. BRASIL. **Amazonas:** Río Jurua-Mirim, Varzea, *Prance et al. 13058* (US). **Roraima:** Sierra Tepecum, sub-base Surucucu, *Rosa & Laureiro 365* (NY). VENEZUELA. **Amazonas:** Margen Caño Asita, Serranía Parú, Río Parú, *Cowan & Wurdack 31531* (VEN). **Bolívar:** norte del Macizo Ichún, Alto Río Paragua, *Hernández 4471* (NY); Campamento Las Pavas, Salto Pará, Río Caura, *Steyermark & Dunsterville 113106* (VEN); selvas al pie de la quebrada Parumeru entre Kavanayén y Río Pacairao, afluente del Río Moak, *Steyermark 60412* (MO, VEN); selvas del Río Curutu, Alto Paragua, *Cardona 2485* (VEN); en rebalses del Río Carún, Alto Paragua, *Cardona 2461* (VEN).

4. Alexa cowanii Yakovlev, Bot. Zurn. 62(3): 436. 1977. TIPO: *Tillett & Tillett 45630* (holótipo, NY). Figura 4.

Arbol 8–10 m alto. Hojas 47–71 cm largo: pecíolo 12–22 cm largo, con pubescencia dispersa adpresa; raquis de terete a semiterete 20–36 cm largo, pubescencia dispersa y acostada; peciólulo 0.4–0.6 cm largo, pubescencia densa o dispersa; folíolos 8–13, opuestos, subopuestos o alternos, membranáceos, con nervios poco prominentes, oblongos-ovobados, color verde claro, (4–)9–18 × (2–)4–9 cm, con pubescencia densa o dispersa de pelos cortos y adpresos abaxialmente, base obtusa, ápice abruptamente largo acuminado. Inflorescencia panícula racemiforme terminal, 15–17 cm largo, con 40–55 flores; eje densamente pubescente, de color rojizo; pedúnculo de inflorescencia 5.2–25.2 cm largo; brácteas pediceladas, ovadas y acuminadas, 0.7–1.2 × 0.2–0.21 cm; bracteólas pediceladas, ovadas y acuminadas, 1.5–10 × 0.1–0.2 mm. Flores 4.5–5.1 cm largo; pedicelo 0.35–1 cm largo; hipanto acampanado, subcoriáceo, 0.5–1 cm largo, con pubescencia externa densa y de color marrón oscuro; cáliz acampanado, subcoriáceo, 1–1.1 cm largo, pubescencia externa densa e internamente glabro con pelos hacia el borde; lóbulos ausentes o 1(–2), 6–7 mm largo o 5–6 apiculos en el borde del cáliz 0.1–0.2 mm largo; vexilo obovado, membranáceo 4.3–5.2 × 2–2.2 cm, glabro o glabrescente externamente, ápice emarginado, bordes lisos; alas de lanceoladas a falcadas, membranáceas, 4.3–4.8 × 0.6–0.7 cm, glabras, ápice agudo, bordes lisos; pétalos carinales lanceolados y falcados, membranáceos, 4.5–4.8 × 0.5–0.6 cm, glabros, ápice agudo, bordes lisos; androceo formado por 10 estambres; filamentos 3.8–4.1 cm largo; anteras 8–11 × 1.5–2 mm; gineceo 3–3.4 cm largo; ovario con pubescencia marrón oscura, 9–14 × 2–3 mm; ginóforo pubescente, 0.25–0.3 cm largo; estilo glabro e incurvo hacia el ápice, 1.5–1.6 cm largo. Fruto 11–14 × 2.2–3.3 cm, con pubescencia densa y marrón, 2–4 semillas. Semillas 2.4–3.1 × 1.2–1.5 cm.

Nombres comunes. Tinajito, Tinajita.

Distribución. En Venezuela al este del Estado Bolívar y en Guyana en las áreas cercanas a la frontera con Venezuela. De acuerdo al mapa de vegetación de Venezuela (Huber & Alarcón, 1988), *Alexa cowanii* se distribuye en bosques siempreverdes.

Alexa cowanii se diferencia de las otras especies por presentar panículas racemiformes, gineceo con estilo y ginóforo más cortos que sus congeneres.

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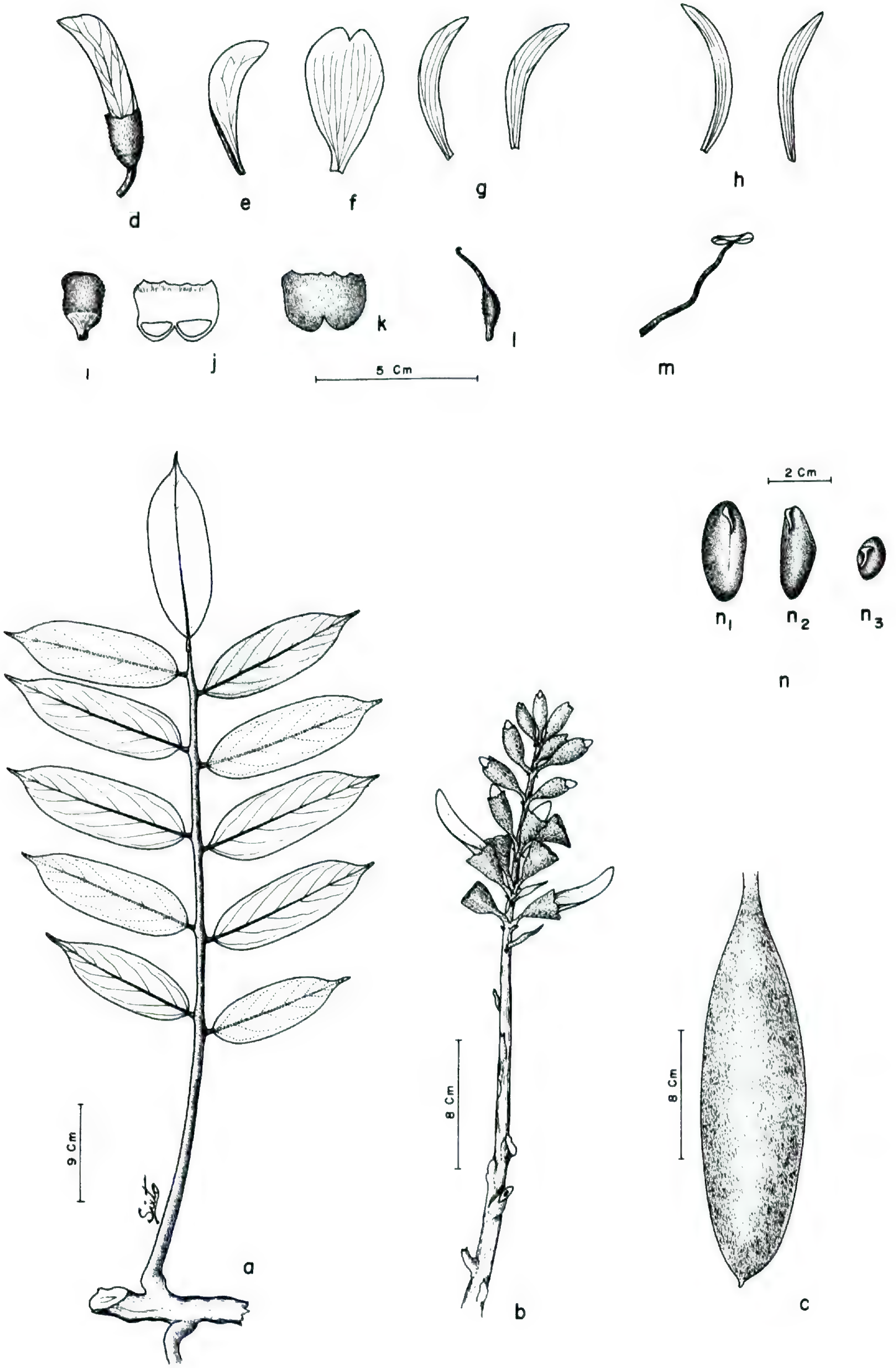
FIGURA 3. *Alexa confusa* Pittier.—a. Hoja.—b. Inflorescencia.—c. Fruto.—d. Flor.—e. Pétalo vexilar.—f. Alas, cara interna y externa.—g. Pétalo carinal.—h. Cáliz.—i. Cáliz desplegado, lado externo.—j. Cáliz desplegado, lado interno.—k. Estambre.—l. Pistilo.—m. Semilla: m_1 vista frontal, m_2 vista lateral, m_3 vista superior.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n1
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9 Cm
a
8 Cm
b
8 Cm
c

Esta especie está más relacionada con *A. canaracunensis* pero difiere en sus flores más pequeñas.

Especímenes examinados. GUYANA. Alto Río Mazaruni, bosque pluvial, *Tillett & Tillett 45706* (US). VENEZUELA. **Bolívar:** entre El Dorado y el caserío del Km 88, a lo largo de las trochas La "Lira" y "Corazón Jesus," *Blanco 604* (VEN).

5. Alexa grandiflora Ducke, Arch. Jard. Bot. Rio de Janeiro I: 33–34. 1915. TIPO: *Ducke 15558* (holótipo, MG no visto). Figura 5.

Arbol 10–35 m alto. Hojas (21–)27–41 cm largo; pecíolo 4–8 cm largo, de pubescente a glabro: raquis terete, (9–)12–18 cm largo, pubescencia escasa; folíolos 5–9, rara vez 7, alternos, rara vez subopuestos, subcoriáceos, con nervios poco prominentes, angostamente elípticos a elípticos, de color verde claro a marrón claro, (6–)10–13(–15) × 3–6 cm, adaxialmente de escasamente pubescente a glabros, abaxialmente con pubescencia escasa sobre los nervios, base obtusa o subaguda, ápice corto-medianamente acuminado. Inflorescencia racimo terminal, rara vez racimo bifurcado, 5–10(–21) cm largo, con 7–23 flores; eje denso o escasamente pubescente, rara vez con lenticelas; pedúnculo de la inflorescencia 1–1.3 cm largo; brácteas triangulares y agudas; bracteólas de triangulares a piramidales, 0.2–0.45 × 0.2–0.4 cm; pedicelo floral 1.7–4.5 cm largo. Flores 1.7–4.5 cm largo; hipanto acampanado, coriáceo, 0.5–0.6 cm largo, pubescencia externa densa, marrón oscura, internamente glabro; cáliz acampanado, coriáceo, 1.2–1.4 cm largo, con pubescencia densa externa e internamente; lóbulos grandes (1–)3–4(–6) × 0.35–0.8 cm largo, pequeños 0.05–0.2 cm largo; vexilo de suborbilar a obovado, de subcoriáceo a coriáceo, 4–4.6 × 2.5–3 cm, pubescencia densa externamente, ápice emarginado, borde liso; alas angostamente obovadas a oblongas, subcoriáceas a coriáceas, 3.5–4.5 × 1–1.1 cm, pubescencia densa externamente, ápice agudo a semiobtuso, bordes lisos; pétalos carinales lanceolados, subcoriáceos a coriáceos, 4–4.5 × 1–1.1 cm, pubescencia densa externamente, ápice ligeramente incurvo, agudo a obtuso, bordes lisos; androceo formado por 10 estambres, (2.3–)3.5–4.1 cm largo, anteras 0.6–0.9(–1.3) × 0.1–0.15 cm; gineceo (2.8–)3.5–5.2 cm largo; ovario con pubescencia densa y amarillenta, 1.2–2.8 × 0.35–0.5 cm; ginóforo glabro, 1–1.5 cm largo; estilo glabro, ligeramente incurvo hacia el ápice, 0.6–1.2 cm largo. Fruto (16–)20–25 × 3.5–4.5 cm, pubescencia densa marrón oscura, pelos cortos, 3 semillas. Semillas 2.5–3 × 2.5–3 cm.

Nombres comunes. Melancieira.

Distribución. Esta especie se distribuye exclusivamente en Brasil, en el estado de Pará, en bosques siempreverdes, asociados o no a la vegetación riparina, sobre suelos inundables o no inundables.

Alexa grandiflora está más relacionada con *A. imperatricis* pero difiere en que sus inflorescencias son terminales y en sus hojas frecuentemente más pequeñas.

Especímenes examinados. BRASIL. **Amapa:** Municipio Macapá, Cupixi 45 Km W-SW de Porto Grande sobre "perimetral norte," *Ravelo & Souza 3235* (NY). **Amazonas:** Borba, Rio Madeira, Selva no inundable, *Ducke 23351* (US). **Pará:** Municipio de Almeirim, Monte Dorado, *Santos 417* (NY); Rio Tocantins, *Ducke 286* (MO, US); Jurupá, *Ducke 15974* (US); Municipio Oriximiná, 18 Km de Cachoeira Br 163, acasso ao perimetral norte, *Ferreira 9725* (NY).

6. Alexa herminiana N. Ramírez, Ernstia 37: 41–43. 1986. TIPO: *Guánchez 2774* (holótipo, VEN). Figura 6.

Arbol 10–20 m alto. Hojas 36–49 cm largo; pecíolo acanalado, 7–12 cm largo, glabrescente; raquis terete, acanalado, 16–23 cm largo, glabrescente; peciólulos 0.8–1.1 cm largo; folíolos subopuestos, membranáceos a subcoriáceos, con nervios poco prominentes, elípticos, ocasionalmente oblongo-obovados, de color verde claro a marrón, (8–)9–15(–18) × 5–8(–9) cm; lamina glabra por ambas caras, ocasionalmente con pelos muy dispersos sobre el nervio central, adpresos, rara vez erectos, base obtusa, ápice abruptamente corto acuminado, ocasionalmente abrupto largo acuminado. Inflorescencia racimo terminal, 10–21 cm largo, con 11–19 flores; eje con pelos dispersos y adpresos, de color rojizo, con lenticelas; pedúnculo de la inflorescencia 3–6.5 cm largo; brácteas sésiles, triangulares y acuminadas, 0.2–0.3(–0.8) × 0.2–0.3 cm, ocasionalmente con brácteas en las flores basales ca. 0.8 cm largo; bracteólas sésiles, ovadas y agudas, 1–1.1 × 0.4–0.8 cm. Flores 5–6 cm largo, glabras; pedicelo terete, acanalado, 4.2–6.5 cm largo; hipanto acampanado a semiturbinado, cori-

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FIGURA 4. *Alexa cowanii* Yakovlev.—a. Hoja.—b. Inflorescencia.—c. Fruto.—d. Flor.—e. Pétalo vexilar.—f. Pétalo vexilar desplegado.—g. Alas.—h. Pétalo carinal.—i. Cáliz.—j. Cáliz desplegado, lado interno.—k. Cáliz desplegado, lado externo.—l. Pistilo.—m. Estambre.—n. Semilla: n_1 vista frontal, n_2 vista lateral, n_3 vista superior.

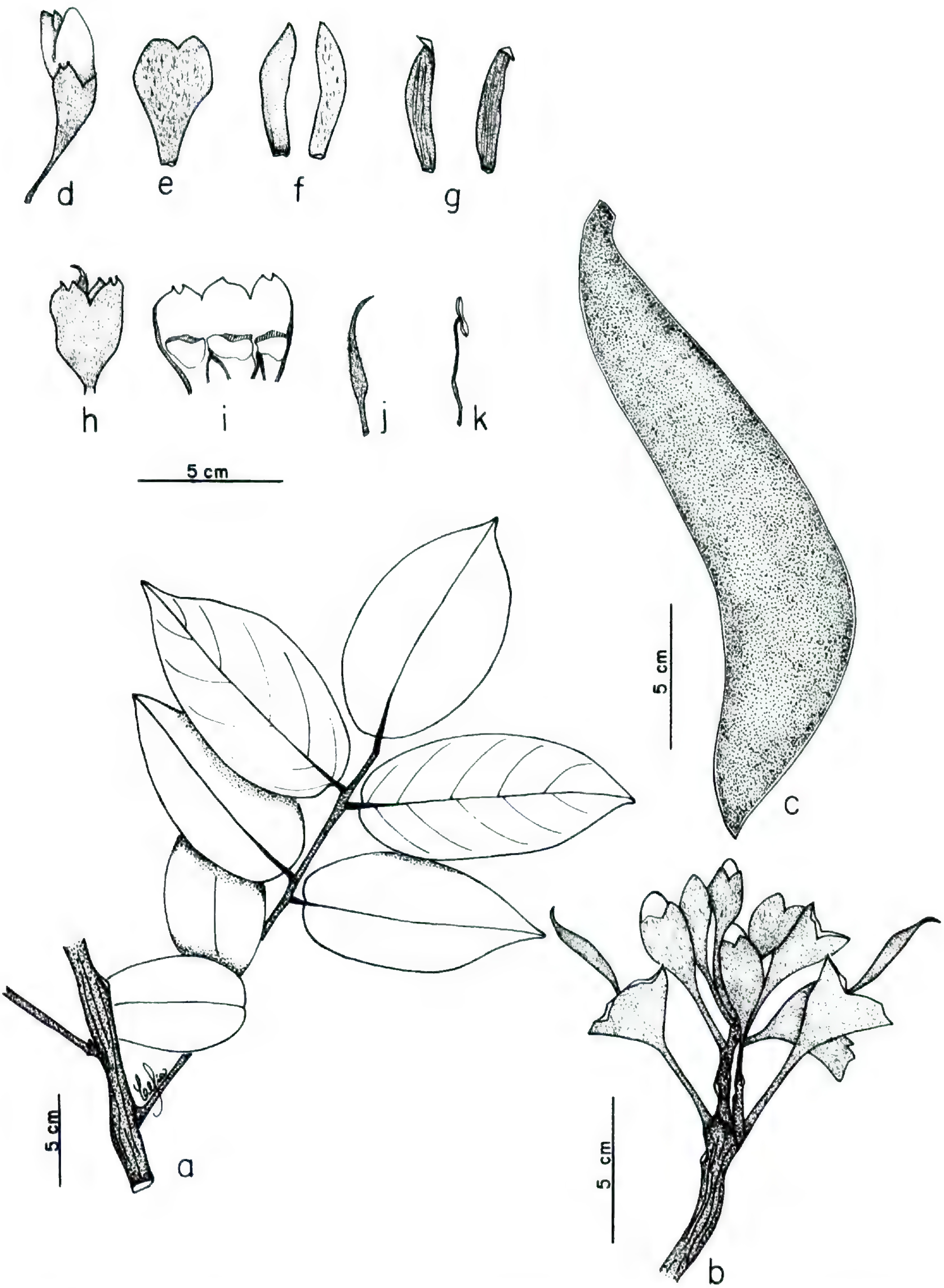

FIGURA 5. *Alexa grandiflora* Ducke.—a. Hoja.—b. Inflorescencia.—c. Fruto.—d. Flor.—e. Pétalo vexilar.—f. Alas.—g. Pétalos carinales.—h. Cáliz.—i. Cáliz desplegado, lado interno.—j. Pistilo.—k. Estambre.

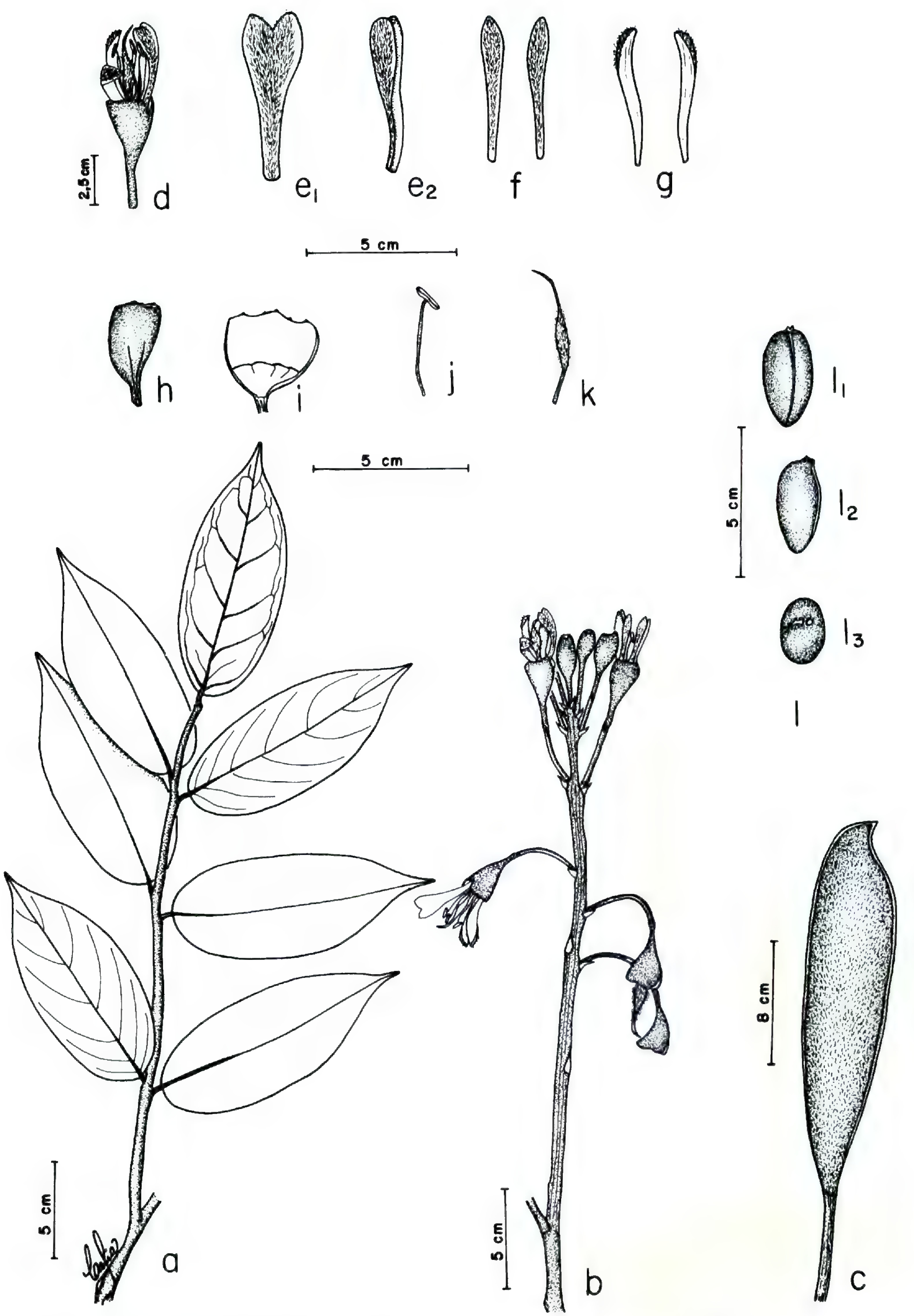

FIGURA 6. *Alexa herminiana* N. Ramírez.—a. Hoja.—b. Inflorescencia.—c. Fruto.—d. Flor.—e. Pétalo vexilar: e_1, pétalo vexilar desplegado; e_2, pétalo vexilar cerrado.—f. Alas.—g. Pétalos carinales.—h. Cáliz.—i. Cáliz desplegado, lado interno.—j. Estambre.—k. Pistilo.—l. Semilla: l_1 vista frontal, l_2 vista lateral, l_3 vista superior.

áceo, 0.7–1.2 cm largo, color marrón oscuro a rojizo, glabro; cáliz acampanado, coriáceo, 1.6–1.7 cm largo, glabro, con un lóbulo grande 0.5–1.1 cm largo y cinco pequeños 1–2.5 mm largo, con ápices redondeados-agudos; vexilo obovado, membranáceo a subcoriáceo, 4.6–5 × 1.8–2.1 cm, pubescencia densa externamente, ápice emarginado, con bordes lisos; alas lanceoladas semiobovadas, membranáceas a subcoriáceas, 4.4–4.5 × 0.6–0.7 cm, pubescencia densa externamente, ápice ligeramente agudo, borde liso, pétalos carinales lanceolados a semiobovados, subcoriáceos, 4.4–4.5 × ca. 0.6 cm, pubescencia densa externamente, ápice ligeramente agudo, bordes lisos; androceo formado por 10 estambres; filamentos 3.3–3.6 cm largo; anteras 0.9–1.1 × 0.3–0.35 cm; gineceo 4.8–5 cm largo; ovario densamente pubescente, marrón amarillento, 1.5–1.6 × 0.3–0.35 cm; ginóforo pubescente, 1.5–1.7 cm largo; estilo glabro, ligeramente incurvo 1.7–1.8 cm largo. Fruto 24–28.5 × 5.5–7.1 cm, pubescente, marrón-ferrugineo, 3–4 semillas. Semillas 2.8–3.7 × 1.4–1.6 cm.

Alexa herminiana se diferencia de sus congéneres por tener el hipanto y cáliz externamente glabros. El epíteto especifico fue una dedicatoria a mi madre Maria Herminia, ahora fallecida.

Distribución. En Venezuela al noroeste y sudoeste del Amazonas y por la cercanía de las áreas de colección probablemente en Colombia y Brasil. De acuerdo al mapa de vegetación de Venezuela (Huber & Alarcón, 1988), *Alexa herminiana* se distribuye en bosques siempreverdes y en bosques ribereños, específicamente en aquellos estacionalmente inundables.

Especímenes examinados. VENEZUELA. **Amazonas:** Departamento Atures, riberas del Río Cataniapo, Raudal Rabipelado, *Guánchez 1006, 2774* (VEN).

7. **Alexa imperatricis** (Schomburgk) Baillon, Hist. Pl. 2: 362. 1870. *Alexandra imperatricis* R. H. Schomburgk, Lond. J. Bot. 4: 12. 1845. TIPO: *Schomburgk 1565* (holótipo, K no visto). Figura 7.

Alexa leiopetala Sandwith, Kew Bull. 228. 1941. TIPO: *Fanshawe 3077* (holótipo, K no visto).
Alexa imperatricis (Schomb.) Baillon var. *glabra* Yakovlev, Bot. Zurn. 62: 433. 1977. TIPO: *Bernardi 7294* (holótipo, NY).

Arbol 30–40 m alto. Hojas (32–)50–92 cm largo; pecíolo 8–17 cm largo, glabro o glabrescente; raquis terete-acanalado, (9–)15–38 cm largo, con pelos dispersos y erectos; peciólulo 0.8 –1.8 cm largo, con pubescencia escasa; folíolos 5–9, alternos a subopuestos, subcoriáceos, con nervios prominentes abaxialmente, elípticos, oblongos u obovados, de color verde claro, (6–)12–34 × (4–)5–13(–15) cm, con pubescencia escasa abaxialmente sobre los nervios, base obtusa, ápice de angosto a abruptamente corto acuminado. Inflorescencia racemosa, creciendo en tallos viejos (caulifloria), 3–14 cm largo, con 6–26 flores; eje densamente pubescente con pelos largos de color marrón oscuro; pedúnculo de la inflorescencia 0.5–3.3 cm largo; brácteas ovadas, acuminadas, 1.5–3 × 1.5–5 mm; bracteólas ovadas, acuminadas, 1–1.5 × 0.2–0.5 mm. Flores 3–5 cm largo; pedicelo 3.2–5 cm largo; hipanto acampanado, coriáceo, 0.7–0.9 cm largo, con pubescencia externa densa, marrón oscura; cáliz acampanado, coriáceo, 1.4–1.9 cm largo, pubescencia externa densa e internamente glabro con pelos esparcidos sobre los lóbulos, lóbulos 3–5, 3–7 mm largo o con pequeños apiculos 1–1.5 mm largo; vexilo suborbicular, coriáceo, 3.1–3.2 × 2.5–2.8 cm, glabro o glabrescente externamente, ápice emarginado, borde liso; alas de obovadas a elípticas, coriáceas, 3.1–3.2 × 1–1.1 cm, glabras, ápice agudo a obtuso, bordes lisos; pétalos carinales de ovobados a elípticos, coriáceos, 3–3.1 × 0.9–1 cm, glabros, ápice de agudo a obtuso, bordes lisos, androceo formado por 8–10 estambres; filamentos 2–4.1 cm largo; anteras 6.5–7 × 1–1.5 mm; gineceo 3.5–3.8 cm largo; ovario con pubescencia marrón a dorada, 1.4–1.5 × 0.3–0.35 cm; ginóforo glabro, 1–1.5 cm largo; estilo glabro, incurvo hacia el ápice, 1–1.5 cm largo. Fruto 19.1–32.2 × 1.8–5.6, pubescencia marrón oscura, glabrescente en la madurez, con 1–4 semillas. Semillas 3–3.2 × 2.3–3.3 cm.

Nombre común. Leche de cochino.

Distribución. En Venezuela al sur y centro del Estado Delta Amacuro, y al nordeste del Estado Bolívar, y en el noroeste de Guyana. De acuerdo al mapa de vegetación de Venezuela (Huber & Alarcón, 1988), *Alexa imperatricis* se distribuye en bosques siempreverdes y semideciduos.

Alexa imperatricis se diferencia de las otras es-

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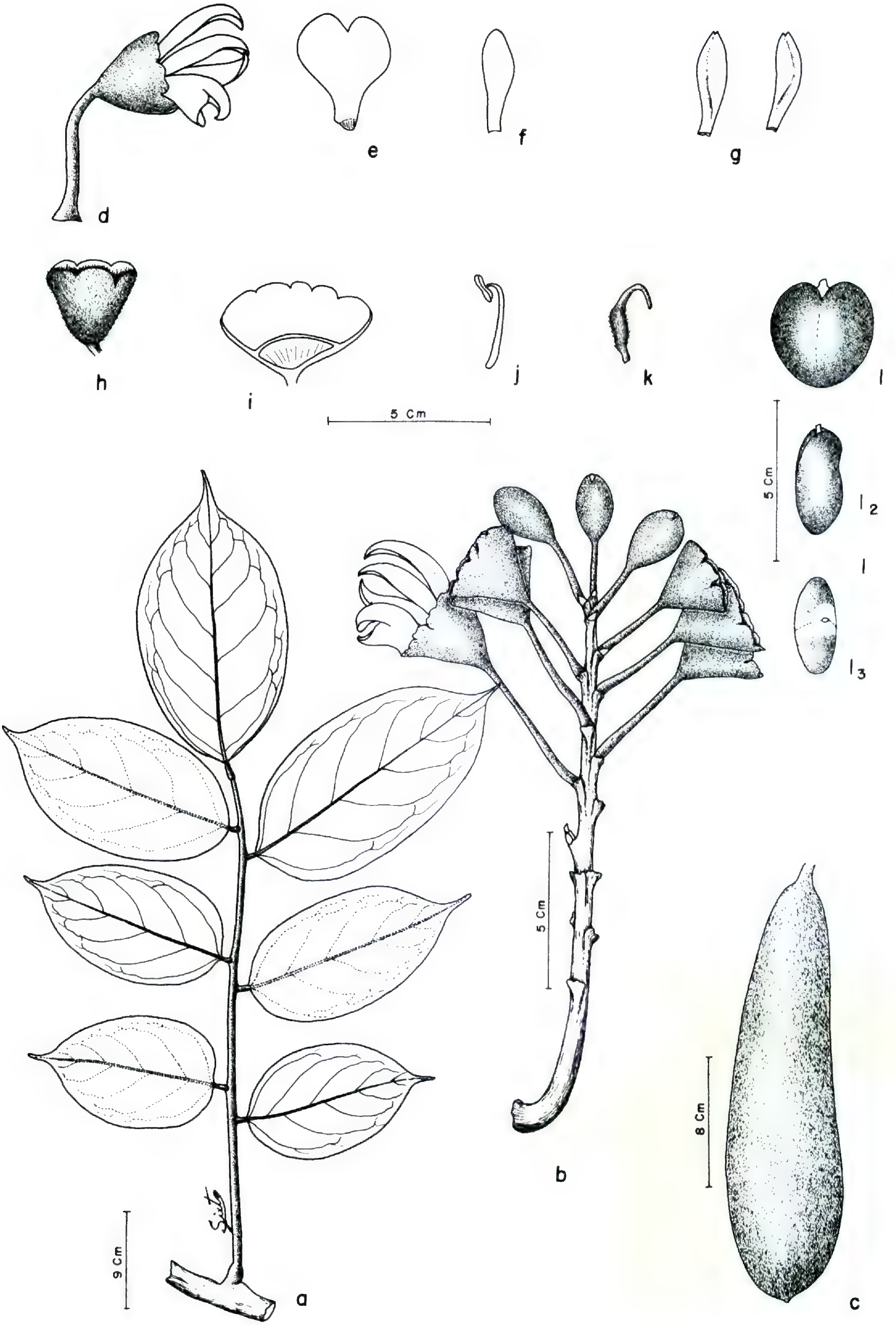
FIGURA 7. *Alexa imperatricis* (Schomb.) Baillon.—a. Hoja.—b. Inflorescencia.—c. Fruto.—d. Flor.—e. Pétalo vexilar.—f. Alas.—g. Pétalos carinales.—h. Cáliz.—i. Cáliz desplegado, lado interno.—j. Estambre.—k. Pistilo.—l. Semilla: l_1 vista frontal, l_2 vista lateral, l_3 vista superior.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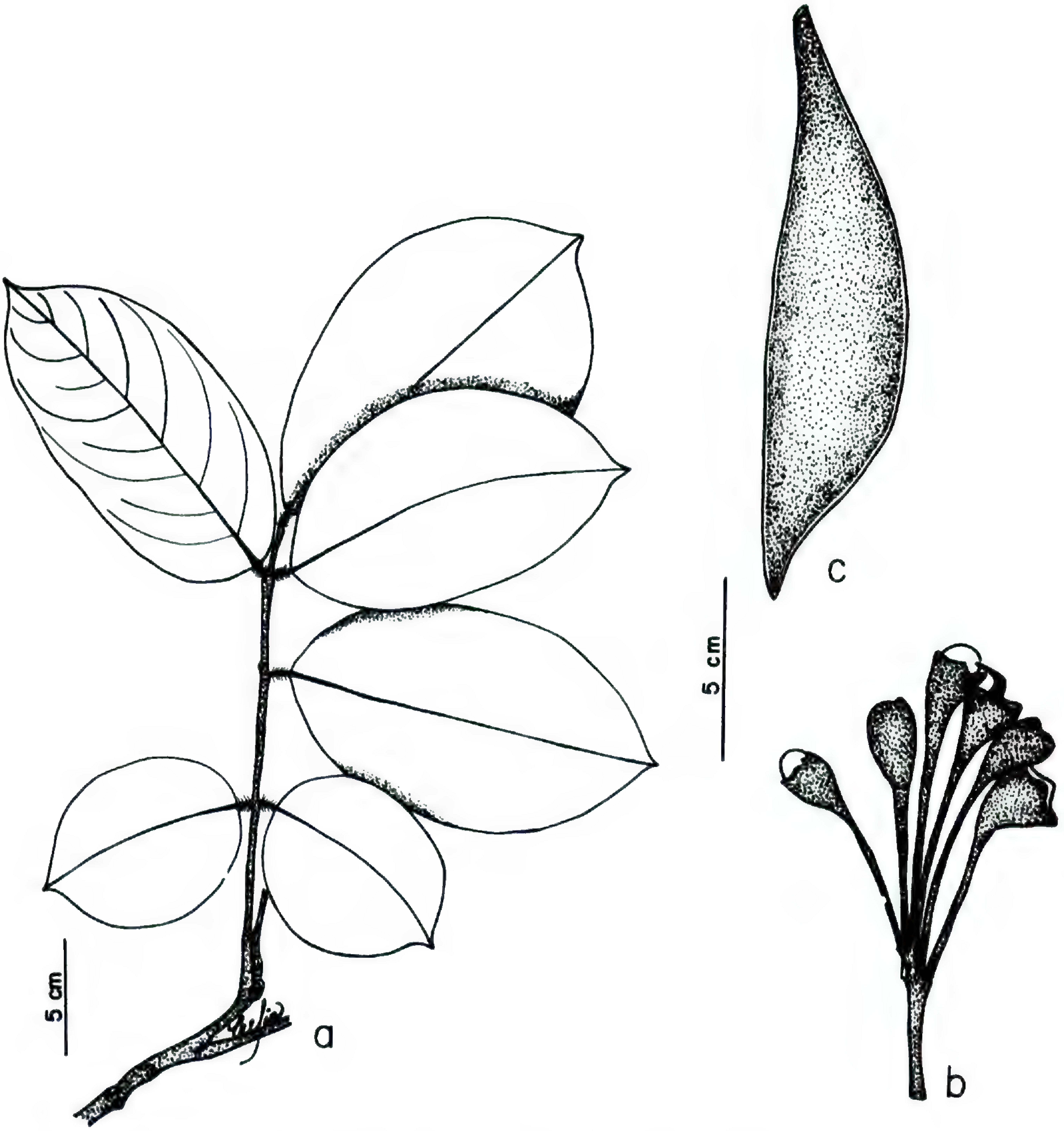

FIGURA 8. *Alexa surinamensis* Yakovlev.—a. Hoja.—b. Inflorescencia.—c. Fruto.

pecies por presentar pétalos vexilares, alas y pétalos carinales, así como el gineceo más cortos que sus congeneres.

Especímenes examinados. GUYANA. Río Pomeroon, Mairaka, *Sandwith 258* (US); Distrito del Noroeste, *Archer 2411* (US); Alto Río Mazaruni, *Tillett & Tillett 45295* (US); región de Barima-Waini, Río Barima, este de Arakaka, *Pipoly & Lall 8042* (NY). VENEZUELA. **Bolívar:** El Palmar, Departamento Piar, La Isabel a Río Grande, *Conejo 31* (VEN). **Delta Amacuro:** Río Amacuro, Sierra de Imataca, *Steyermark 87344* (NY, US, VEN); Delta del Río Orinoco, *Suárez 2* (VEN); Distrito Sifontes, Concesión Minera Cristina 4, 16 Km al noroeste del Km 88, *Aymard et al. 4077* (MO).

8. Alexa surinamensis Yakovlev, Bot. Zurn. 62(3): 437. 1977. TIPO: *Maguire, Schulz, Soderstrom & Holmgren 54017* (holótipo, NY). Figura 8.

Arbol 12–20 m alto. Hojas (31–)38–61 cm largo, pubescencia escasa; pecíolo 9–15 cm largo, pubescencia de dispersa a escasa; raquis terete, 11–14 cm largo, pubescencia escasa; peciólulo 0.7–0.9 cm largo, pubescencia escasa y dispersa; folíolos 5–7, opuestos a subopuestos, membranáceos a subcoriáceos, con nervios poco prominentes abaxialmente, angostamente elípticos a elípticos, rara vez ovados-ampliamente obovados, de color verde oscuro a marrón verdoso, (9–)17–31 × (7–)11–16 cm, glabros adaxialmente, con pubescencia escasa

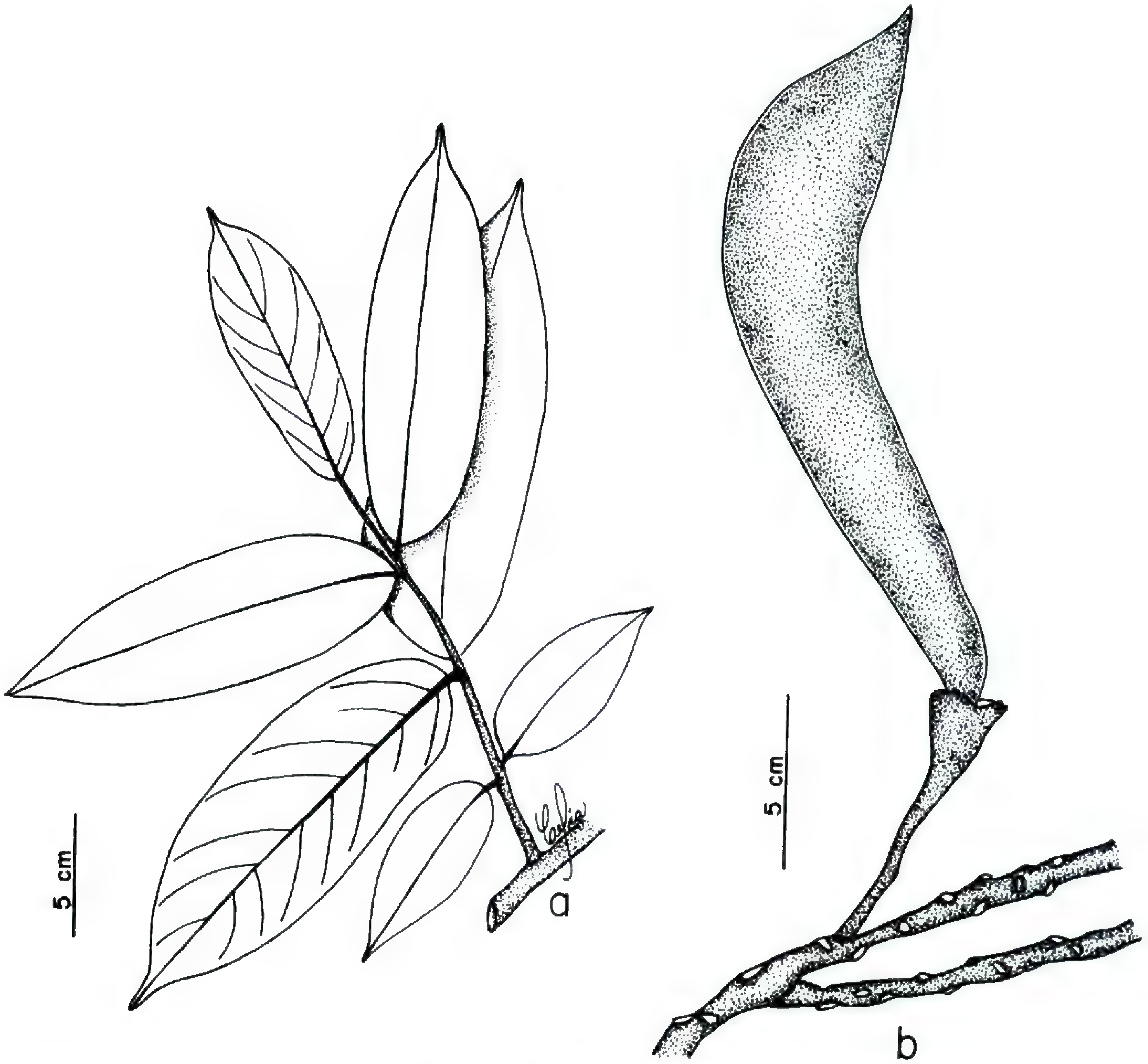

FIGURA 9. *Alexa wachenheimii* Benoist.—a. Hoja.—b. Fruto.

abaxialmente sobre los nervios, base obtusa a aguda, ápice largo-corto acuminado, rara vez abrupto largo acuminado. Inflorescencia racemosa creciendo sobre tallos viejos (caulifloria), ca. 7.8 cm largo, con ca. 8 flores; eje densamente pubescente; pedúnculo de la inflorescencia ca. 3.3 cm largo; bracteólas triangulares. Flores ca. 3.2 cm largo; pedicelo 4–9 cm largo; hipanto acampanado, coriáceo, con pubescencia externa densa e internamente glabro; cáliz acampanado, coriáceo, pubescencia densa externamente e internamente escasa; lóbulos 4 grandes, 0.35–0.4 cm largo; estilo glabro, ca. 1 cm largo. Fruto densamente pubescente, ca. 30 cm largo con 3 semillas. Semillas ca. 2.5 × 2.3 cm.

Nombres comunes. Muellera, Haiariballi (Arawak).

Distribución. *Alexa surinanensis* se distribuye en bosques mesofíticos asociada a riparios, en Guyana y Surinam.

Alexa surinamensis se diferencia de sus congéneres por presentar folíolos membranaceos, más anchos y desde angostamente elípticos a ampliamente ovados-obovados en comparación con las otras especies.

Especímenes examinados. GUYANA. Distrito noroeste de Hossororo: *Archer 2259* (US). SURINAM. 2 Km arriba de la confluencia del Río Lucie, 220 m de altitud, bosque mesofítico, *Maguire et al. 54017* (US).

9. Alexa wachenheimii Benoist, Bull. Mus. Hist. Nat. (Paris) 27: 115. 1921. TIPO: *Wachenheim 93* (holótipo, P). Figura 9.

Arbol (11–)20–35 m alto. Hojas 30–58 cm largo; pecíolo 8–11 cm largo, glabrescente a glabro; raquis terete acanalado, 14–27 cm largo, peciólulo 0.5–0.9 cm largo, glabrescente a glabro; folíolos 7–9, alternos, rara vez subopuestos, subcoriáceos a

coriáceos, con nervios poco prominentes, angostamente elípticos a elípticos, rara vez obovados-oblongos, de color verde claro a marrón verdoso, (6–)8–20(–23) × (3–)4–8(–9) cm, adaxialmente glabros, abaxialmente con pubescencia escasa sobre los nervios, base obtusa a redondeada, algunas veces aguda, ápice angosto-largo acuminado, rara vez agudo. Inflorescencia racimo o racimo 2-bifurcado, terminal, 10–20 cm largo, con 7–53 flores, eje glabro a glabrescente, rara vez con lenticelas; pedúnculo de la inflorescencia 6–7 cm largo; brácteas triangulares, 4–6 × 4–5.5 mm largo; bracteólas triangulares, algo piramidales, 3–5 × ca. 5 mm. Flores 4.3–5.6 cm, pedicelo floral 2.2–4.5 cm largo; hipanto acampanado, coriáceo, con pubescencia externa densa, marrón oscura, glabro internamente; cáliz acampanado, coriáceo, 1.8–2.5 cm largo, con pubescencia densa externamente, escasa internamente, con pelos glandulares; lóbulos 3 grandes, ca. 0.5–0.6 cm largo; vexilo 4–4.5 × 1.8–3 cm, pubescencia densa externamente; alas triangulares-ovoides a lanceoladas, ca. 4–5 × 0.8 cm, pubescencia densa externamente; pétalos carinales oblongos, ca. 4–5 × 0.6–0.8 cm, pubescencia densa externamente; gineceo ca. 3 cm largo, ovario densamente pubescente, ca. 1 cm largo, ginóforo glabro, ca. 1 cm largo, estilo glabro ca. 1 cm largo. Fruto (13–)18–25 cm largo, marrón castaño, densamente pubescente, con 3–4 semillas. Semillas 2–3 × 1.5–2 cm.

Nombres comunes. Nekoe hoedoe, Arromatta belero, Korrekoran.

Distribución. *Alexa wachenheimii* se distribuye en Guyana, Guayana Francesa y Surinam, generalmente en bosques siempreverdes asociados a cursos de agua.

Alexa wachenheimii se diferencia de sus congéneres por presentar inflorescencias con brácteas de 4–6 × 4–5.5 cm y bracteólas 3–3.5 × 3–5 cm. Flores con alas 0.8 cm ancho y cáliz de 1.5 cm largo.

Especímenes examinados. GUYANA FRANCESA. Ensenada Armontao en la confluencia del Oyapoc, *Prevost & Grenand 2027* (US); Rivera derecha del Río Yaraupi, 4 Km hacia la desembocadura, *Oldeman B-3121* (US). SURINAM. Watramiri, *B. W. 4220* (US), *4308* (US); en los margenes del Río Perica, *Lindeman 5311* (VEN). GUYANA. Río Essequibo, Madray, *Anónimo* (NY).

Resultados Anatómicos

Clave Anatómica Para las Especies Venezolanas del Género *Alexa*

1. Parénquima esponjoso con células irregulares o rectangulares, alargadas anticlinalmente; células de la epidermis superior con 4–5(–6) lados en vista superficial 2
1. Parénquima esponjoso con células irregulares o redondeadas; células de la epidermis superior cuadrangulares en vista superficial 3
2(1). Pelos adpresos 37.5–72.5 μm largo, glandulares pluricelulares, unicelulares no glandulares .... *A. imperatricis*
2. Pelos erectos 14–17 μm largo, con 1–3 células .... *A. herminiana*
3(1). Parénquima de empalizada con 1 capa de células; parénquima esponjoso con células circulares o redondeadas; cutícula adaxial 0.7–2.5 μm de grosor y cutícula abaxial 0.2–0.4 μm de grosor 4
3. Parénquima empalizada con 1(–2) capas de células; parénquima esponjoso con células irregulares; cutícula adaxial 2.5–7.5 μm de grosor y cutícula abaxial 0.5–2.5 μm de grosor 5
4(3). Pelos adpresos 27–67 μm largo; epidermis superior 7–10 μm de grosor .... *A. cowanii*
4. Pelos erectos 87–165 μm largo; epidermis superior 10–12 μm de grosor .... *A. canaracunensis*
5(3). Pelos en ambas caras de la hoja; parénquima esponjoso con 5 capas de células, 32–55 μm de grosor .... *A. confusa*
5. Pelos sólo en la cara abaxial; parénquima esponjoso con 6–8 capas de células, 54–127 μm de grosor .... *A. bauhiniiflora*

Alexa bauhiniiflora. Hoja bifacial, 107–219 μm de grosor; cutícula adaxial ligeramente engrosada, 2.5–6 μm; cutícula abaxial poco engrosada, 1.5–2.5 μm; epidermis adaxial uniestratificada, 15–32 μm, células cuadrangulares, en vista superficial presentan paredes anticlinales ligeramente gruesas, levemente onduladas (Fig. $10f_1$); epidermis abaxial uniestratificada, 10–12.5 μm, células cuadrangulares de bordes curvos, con paredes ligeramente engrosadas e irregulares, en vista superficial presentan paredes engrosadas y ligeramente onduladas (Fig. $10f_2$); estomas anisocíticos; parénquima en empalizada 15–32 μm, formado por 1(–2) capas de células alargadas de extremos curvos y de paredes delgadas (Fig. $10f_3$); parénquima esponjoso 54–127 μm, formado por 6–8 capas de células irregulares, de paredes delgadas con espacios intercelulares (Fig. $10f_3$). Pelos abundantes en la epidermis abaxial, largos y erectos, pluricelulares con 4–8 células y 250–350 μm de longitud (Fig. $10f_4$).

Alexa canaracunensis. Hoja bifacial, 64–98 μm de grosor; cutícula adaxial ligeramente engrosada, 0.7–1.5 μm; cutícula abaxial muy delgada, 0.2–0.5 μm; epidermis adaxial uniestratificada, 11–20 μm, células rectangulares o cuadrangulares, en vista superficial presentan paredes anticlinales delgadas y levemente onduladas (Fig. $10d_1$); epidermis abaxial uniestratificada, 10–12.5 μm, células circulares o

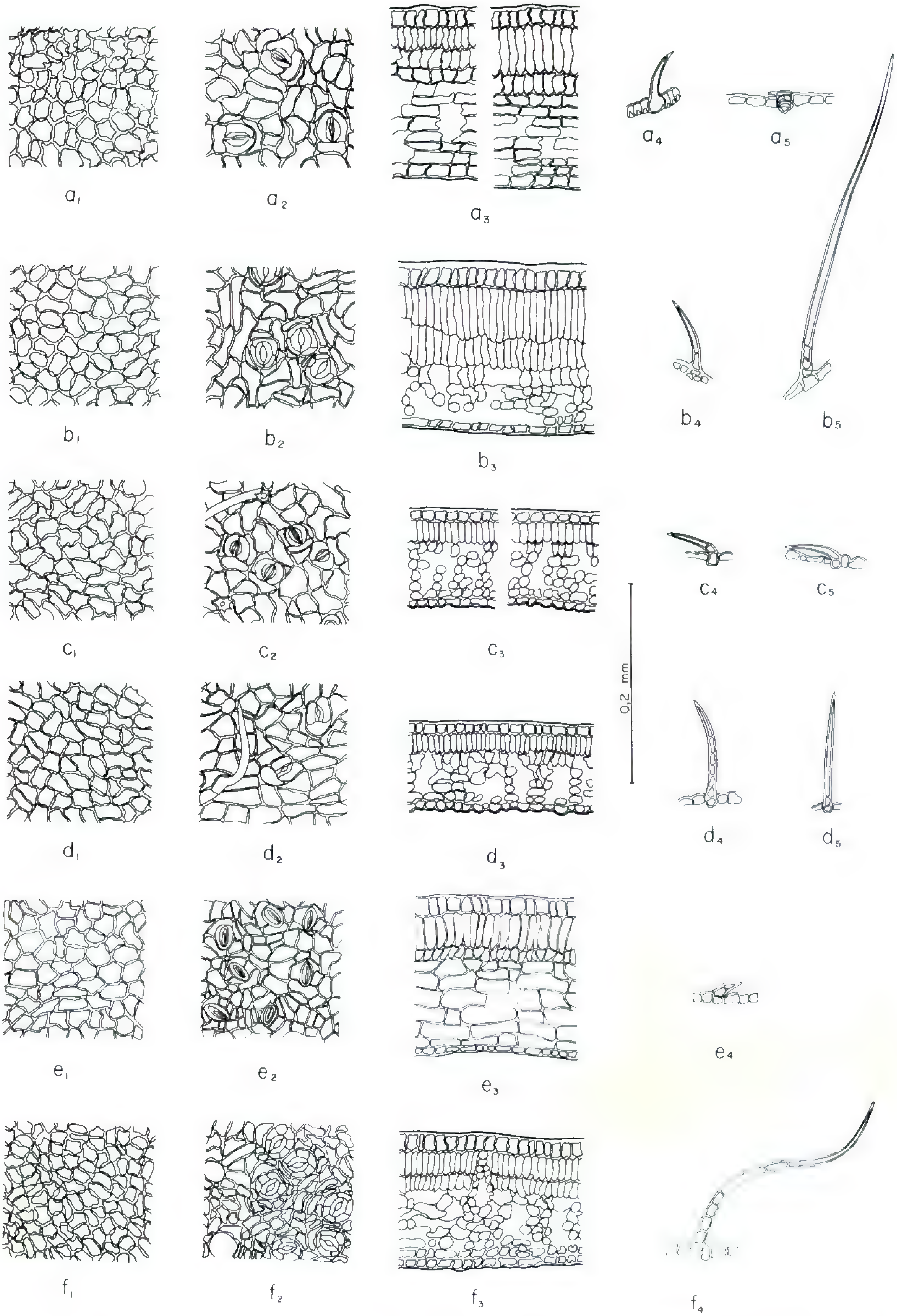

FIGURA 10. Representación de la anatomía foliar para las especies Venezolanas del genero *Alexa*.—a. *A. imperatricis*.—b. *A. confusa*.—c. *A. cowanii*.—d. *A. canaracunensis*.—e. *A. herminiana*.—f. *A. bauhiniiflora*. Subíndices: 1, epidermis superior; 2, epidermis inferior con los estomas; 3, secciones transversales; 4 y 5, tipos de pelos.

redondeadas de paredes delgadas e irregulares, en vista superficial presentan paredes delgadas y rectas (Fig. $10d_2$); estomas anisocíticos; parénquima en empalizada 15–20 μm, formado por una capa de células alargadas con paredes delgadas (Fig. $10d_3$); parénquima esponjoso 37–55 μm, formado por 5(–6) capas de células irregulares con paredes delgadas y espacios intercelulares (Fig. $10d_3$). Pelos en la epidermis abaxial, abundantes, largos y erectos, pluricelular con 3–8 células y 87–165 μm de longitud (Fig. $10d_{4,5}$)

Alexa confusa. Hoja bifacial, 94–160 μm de grosor; cutícula adaxial engrosada, 2.5–7.5 μm; cutícula abaxial más delgada, 0.5–2.5 μm; epidermis adaxial uniestratificada, 11.5–20 μm, células rectangulares o cuadrangulares, en vista superficial presentan paredes engrosadas y levemente onduladas (Fig. $10b_1$); epidermis abaxial uniestratificada, 7.5–10 μm, células rectangulares o cuadrangulares de paredes ligeramente engrosadas e irregulares, en vista superficial presentan paredes delgadas y onduladas (Fig. $10b_2$); estomas anisocíticos; parénquima en empalizada 32.5–77.5 μm, con dos capas de células alargadas de paredes delgadas, siendo la primera de mayor longitud (Fig. $10b_3$); parénquima esponjoso 32–55 μm, formado por 5 capas de células irregulares de paredes delgadas y con espacios intercelulares (Fig. $10b_3$). Pelos abundantes, largos y erectos en ambas caras, escasos sobre la cara adaxial, pluricelulares (1–)2–9 células, 100–362 μm de longitud (Fig. $10b_4$).

Alexa cowanii. Hoja bifacial, 80–93 μm de grosor; cutícula adaxial ligeramente engrosada, 0.7–2.5 μm; cutícula abaxial muy delgada, 0.2–0.4 μm; epidermis adaxial uniestratificada, 7.5–1-μm, células cuadrangulares, en vista superficial presentan paredes anticlinales delgadas y onduladas (Fig. $10c_1$); epidermis abaxial uniestratificada 10–12.5 μm, células circulares o redondeadas, con paredes delgadas e irregulares, en vista superficial presentan paredes engrosadas y levemente onduladas (Fig. $10c_2$); estomas anisocíticos; parénquima en empalizada 12.5–23.7 μm, formado por una capa de células en forma de embudo, de paredes delgadas (Fig. $10c_3$); parénquima esponjoso 32.5–52.5 μm, formado por 5 capas de células irregulares, de paredes delgadas con espacios intercelulares (Fig. $10c_3$). Pelos en la epidermis abaxial, abundantes, cortos y adpresos, pluricelulares, con 2–4 células y 27–67 μm de longitud (Fig. $10c_{4,5}$).

Alexa herminiana. Hoja bifacial, 94.2–220 μm de grosor; cutícula adaxial engrosada, (1.2–)2.5–7.5 μm; cutícula abaxial más delgada, (0.5–)2.5–3.7 μm; epidermis adaxial uniestratificada, 12.5–25 μm, células de cuadrangulares a ligeramente rectangulares, en vista superficial presentan paredes anticlinales ligeramente engrosadas y ligeramente onduladas, de 4–5(–6) lados (Fig. $10e_1$); epidermis abaxial uniestratificada, (0.5–)1.3–3.7 μm, células ligeramente rectangulares, con paredes delgadas, en vista superficial presentan paredes ligeramente engrosadas, de ligeramente redondeadas a alargadas (Fig. $10e_2$); estomas anisocíticos; parénquima en empalizada 17.5–50 μm, formado por células alargadas, de paredes delgadas (Fig. $8e_3$); parénquima esponjoso 52.5–137.5 μm, formado por 7–8 capas de células rectangulares o irregulares, alargadas anticlinalmente, de paredes delgadas con espacios intercelulares (Fig. $10e_3$). Pelos muy escasos y erectos, sobre los nervios más prominentes, pluricelulares con 1–3 células, de 15–17.5 μm de longitud (Fig. $10e_4$).

Alexa imperatricis. Hoja bifacial, 152.5–191 μm de grosor, cutícula adaxial engrosada, 2.5–5 μm; cutícula abaxial más delgada, 0.5–3.7 μm; epidermis adaxial uniestratificada, 12.5–17.5 μm, células cuadrangulares, en vista superficial las células angulares con 4–5 lados, frecuentemente isodiametricas, con paredes anticlinales de onduladas a rectas (Fig. $10a_1$); epidermis abaxial uniestratificada, 5–11 μm, células cuadrangulares e irregulares, con paredes ligeramente engrosadas, en vista superficial presentan paredes delgadas y levemente onduladas (Fig. $10a_2$); estomas anisocíticos; parénquima en empalizada 17.5–47.5 μm, formado por 1–2 capas de células alargadas o cortas de paredes delgadas; parénquima esponjoso 82–112.5 μm, formado por 7–8 capas de células rectangulares o irregulares, y alargadas anticlinalmente, de paredes delgadas o ligeramente engrosadas con espacios intercelulares (Fig. $10a_3$). Pelos en la epidermis abaxial, escasos, cortos y adpresos, unicelulares (Fig. $10a_4$) o pluricelulares de tipo glandular, 37.5–72.5 μm de longitud (Fig. $10a_5$).

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APPENDICE 1. Lista de especies reconocidas.

1. *Alexa bauhiniiflora* Ducke
2. *Alexa canaracunensis* Pittier
3. *Alexa confusa* Pittier
4. *Alexa cowanii* Yakovlev
5. *Alexa grandiflora* Ducke
6. *Alexa herminiana* N. Ramírez
7. *Alexa imperatricis* (Schomb.) Baillon
8. *Alexa surinamensis* Yakovlev
9. *Alexa wachenheimii* Benoist

APPENDICE 2. Especímenes estudiados. Los números entre paréntesis corresponden a los números en la lista precedente y en el texto.

Anónimo (9); Archer 2259 (8), 2270 (8), 2411 (7), 2867 (8); Aymard 3916 (2), 4072 (7), 4077 (7), 4867 (3), 6698 (2).

B. W. 4220 (9), 4308 (9); Bernardi 899 (3), 1611 (3), 2117 (7), 2730 (2), 3026 (4), 6941 (2), 7494 (7); Blanco 604 (4); Boer 2076 (7); Breteler 3881 (7); Burandt 1147 (2); Buza 7212 (7).

Cardona 240 (2), 1473 (1), 1494 (1), 1710 (3), 1913 (3), 2386 (2), 2461 (3), 2485 (3), 2494 (2); Conejo 31 (7); Couset 34 (4); Cowan 31531 (3).

Daly 1332 (5); Delgado 1071 (3); Ducke 46 (1), 286 (5), 15974 (5), 23351 (5).

Fanshawe 2094 (7); Ferreira 9341 (1), 9725 (5); Fróes 22528 (1).

Gentry 14932 (7); Grenand 774 (9); Guánchez 1006 (6), 2774 (6).

Hernández 4471 (3); Holst 2442 (2), 2444 (2), 2447 (2), 2756 (3).

Kubitzki 199 (1).

Lewis 1683 (2), 1689 (2); Liesner 13942 (3), 15887 (1), 19127 (2), 19491 (2); Lindeman 5311 (9).

Marcano-Berti 79 (7), 175 (7), 206 (7); Maguire 54017 (8), 60070 (1).

Oldeman B-3121 (9).

Pinkus 171 (7); Pipoly 6824 (1), 8042 (7); Prance 4441 (2), 9735 (3), 10640 (3), 10841 (2), 13058 (3), 15298 (1), 15600 (1), 15719 (1), 15822 (1); Prevost 2027 (9).

Ramírez 401 (7), 423 (2), 424 (2); Ravelo 3235 (5); Rodriguez 10561 (1), 10500 (1); Rosa 365 (3).

Sandwich 258 (7); Santos 417 (5); Sobrevila 278 (2); Sousa 371 (5); Stergios 3319 (7), 10338 (3), 11788 (2); Steyermark 53510 (4), 60412 (3), 74729 (3), 75425 (2), 86614 (7), 87344 (7), 86706 (4), 87567 (7), 87932 (7), 90634 (3), 107035 (3), 113106 (3), 122206 (6); Suárez 2 (7).

Tillett 45295 (7), 45706 (4); Thomas 3180 (1).

Williams 11402 (3).

A STUDY OF THE VEGETATION AND FLORISTIC AFFINITY OF THE LIMESTONE FORESTS IN SOUTHERN AND SOUTHWESTERN CHINA[1]

Xu Zhaoran[2]

ABSTRACT

Four thousand two hundred eighty-seven specific and infraspecific taxa in 1213 genera and 195 families of vascular plants are reported from the forests occurring on limestone outcrops in southern and southwestern China; the majority are from the provinces of Guangxi, Guizhou, and Yunnan. Data of natural conditions in the explored area, including geology, geography, meteorology, soil sciences, and vegetation, are presented. The flora is treated in 14 distribution types, for example, tropical elements total 69.1% of the flora, northern temperate 9.9%, East Asian 9.9%, and endemics of China 4.7%. Some genera, such as *Camellia*, *Eurya*, *Hydrangea*, *Rhododendron*, and *Symplocos*, are poorly represented in the limestone forests, while *Chirita*, *Excentrodendron*, *Paraboea*, *Platycarya*, and some other genera are endemic or very abundant in this kind of vegetation. About one-third of the species are endemic or dominant species in the limestone vegetation. Characterized by calciphiles, a "limestone flora" is a valid floristic category distinct from non-limestone floras, especially an acid-soil flora in the same region.

The flora of southern and southwestern China is well known for its richness in endemism and biodiversity (Wu, 1980), but the forests occurring on the limestone outcrops in the same region, known as limestone forests, are much less known in the literature. Wu (1980) reported some studies on the plant communities occurring on the limestone substrates in China, but no comprehensive surveys of the vegetation and flora of the limestone forests for the entire southern and southwestern portion of China were available before the present study began.

The current study was carried out from 1982 until 1987 as partial fulfillment of my master's and doctoral degrees at Sun Yatsen University in China, and it continued until recently with funding support from the World Wide Fund for Nature International and the Smithsonian Institution. Data summarized in this paper mostly were collected on my expeditions made from 1982 to recent years (Table 1), which resulted in about 8500 plant collections from forests occurring on limestone substrates, as well as a number of samples of rock and soil, and other data. A checklist of vascular plant species from Chinese limestone forests based on my collections and the literature was compiled (Xu, 1993).

The floristic analysis reported here is based on the published checklist (Xu, 1993). Although my surveys focused on the provinces of Guangxi, Guizhou, and Yunnan, the study results are representative of Chinese limestone forests because these three provinces contain typical Chinese karst landscapes (Fig. 1).

Limestone species are considered for this study to be those that occur on limestone substrates but may also occur on non-limestone substrates; limestone endemic species are those that occur exclusively on limestone substrates.

HABITAT CONDITIONS

China possesses the largest limestone area in the world, with pure carbonate substrates covering an area of 283,000 km² (Fig. 1). The majority of limestone outcrops related to this study lie on the slope from the Guangxi Basin to the Yungui Plateau, i.e., southwestern Guangxi, southern Guizhou, and southeastern Yunnan (Fig. 1, upper left corner),

[1] I am grateful to Chang Hungta for his inspiring suggestion that I pursue this subject in my graduate studies, and to colleagues who directly or indirectly helped improve the current paper. World Wide Fund for Nature International and the Smithsonian Institution provided additional funding support.

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Table 1. Major limestone areas in Southern and Southwestern China surveyed.*

Province	Locality	Coordinate	Vegetation
Guangdong	Lianxian	24°47′N, 112°25′E	Secondary
	Yangshan	24°29′N, 112°41′E	Secondary
Guangxi	Baishe	23°54′N, 106°38′E	Secondary
	Jingxi	23°09′N, 106°25′E	Primary
	Liuzhou	24°29′N, 109°25′E	Secondary
	Longlin	24°46′N, 105°20′E	Primary
	Nandan	24°58′N, 107°33′E	Primary
	Ningming	22°07′N, 107°02′E	Primary
Guizhou	Anlong	25°08′N, 105°27′E	Primary
	Anshun	26°15′N, 105°55′E	Secondary
	Ceheng	25°00′N, 105°48′E	Primary
	Lipo	25°26′N, 107°52′E	Primary
	Xingyi	25°05′N, 104°53′E	Secondary
Yunnan	Funing	23°36′N, 105°36′E	Primary
	Guangnan	24°04′N, 105°04′E	Secondary
	Loping	24°52′N, 104°16′E	Secondary
	Maguan	25°00′N, 104°20′E	Primary
	Wenshan	23°22′N, 104°12′E	Secondary
	Xichou	23°26′N, 104°38′E	Primary

* Only those surveys in which Xu Zhaoran participated (1982–1993). There may be other kinds of vegetation around the locality that were not known to the surveyor(s). The primary vegetation was forest, but the secondary vegetation may be forests or thickets.

which accounts for 198,000 km^2 of pure limestone outcrops (70% of the total in China). Beautiful karst landscapes and numerous fantastic caves provide various ecological niches for plants and wildlife, as well as for tourism.

Chemical composition and geological ages of the carbonate substrates in China are summarized in Lu (1965). The substrates, mainly limestone and partly dolomite, are deposits from the Devonian to the Triassic, ca. 3000–50000 m thick.

Rock samples collected during my expeditions (Table 1) were analyzed in Guangzhou Metallurgical Institute (Guangzhou), and the results show the following composition: calcium oxide (CaO) 50.00% of the total mass, magnesium oxide (MgO) 1.00%, aluminum (Al) trace value (less than 0.005%), manganese (Mn) 0.05%, silicon (Si) 0.01%, and iron (Fe) 0.10%. The above results indicate that the samples are limestone rather than dolomite, according to the classification of Lu (1965).

No contiguous soil layers have been found on pure limestone outcrops or karst hills, and it is unlikely that such soil layers can be formed there. According to Wei (1983), forming a 1-cm-thick soil layer on pure limestone substrates may take 13,000–32,000 years without accounting for leaching or erosion by rain. Because southern and southwestern China have a relatively high annual rainfall, and leaching or erosion is severe, soil deposits can only be found in crevices and pockets of rocks. More surface soil may be retained in limestone hills that are covered by forests than in those without forest cover.

Table 2 shows the chemical composition of some limestone soils. In subtropical areas under virgin forests (Number 3, Table 2), soil from rock crevices is black, with a high concentration of calcium (Ca^{+2}) but low aluminum (Al^{+3}), and the pH is around neutral (6–7). Soil collected from tropical localities with higher precipitation (Number 1, Table 2) may have lower pH values and lower concentration of Ca^{+2}. White soil (Number 2, Table 2) scraped from cliff surfaces has a pH value of 8–9, regardless of whether it was collected from a tropical area.

Some climate data are given in Table 3. The figures were gathered from local meteorological observatories in major collecting sites. In China, areas with an annual ≥10°C accumulated temperature[3] above 6500°C may develop tropical rainforests, and areas with an annual ≥10°C accumulated temper-

[3] The annual ≥10°C accumulated temperature is the total amount of temperature compounded from all the daily temperatures that are higher than or equal to 10 degrees Celsius in one year. This system is used in agriculture, forestry, and botanical research in China because a temperature of 10°C or above is considered to be effective for plant growth.

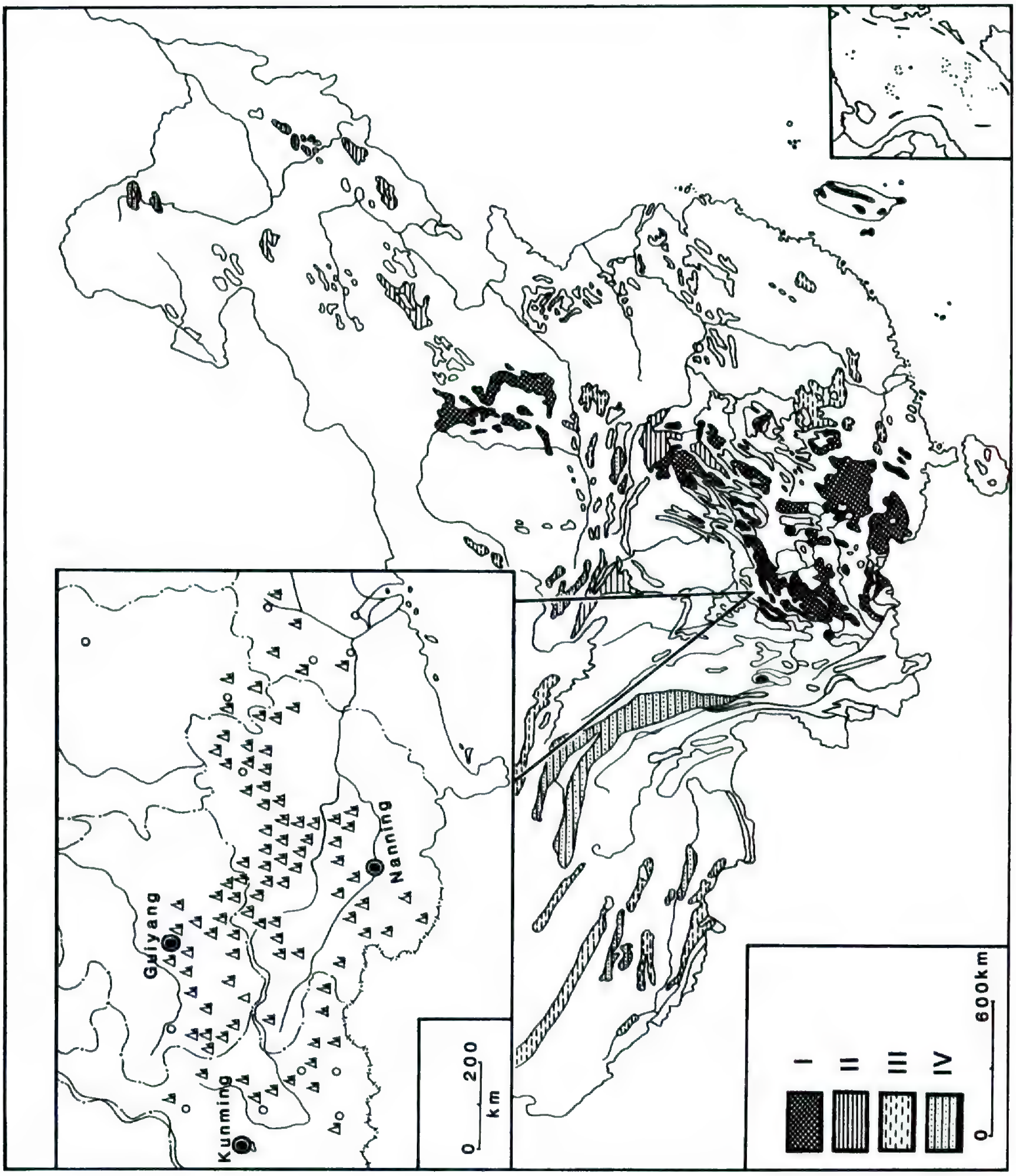
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Table 2. Chemical composition of some limestone soil in Southern and Southwestern China.*

Climate zones	Vegetation and soil types	pH	% of dry weight Mn	Fe	Mg	K	Ca	Al	Zn	Cu	P
Tropical	Number 1: brown soil (secondary forest)	5–6	0.46	9.24	0.45	0.46	0.68	12.0	0.04	0.01	0.26
	Number 2: white soil (on cliffs)	8–9	0.01	0.20	0.13	0.05	8.56	0.36	0.00	0.00	0.00
Subtropical	Number 3: black soil (primary forest)	6–7	0.15	2.20	0.39	0.29	1.38	2.17	0.02	0.00	0.02
	Number 4: brown soil (secondary shrubs)	5–6	0.06	7.65	1.30	0.29	0.37	13.5	0.02	0.00	0.09

* Soil samples were collected by Xu Zhaoran: Number 1, from Ningming, Guangxi; Number 2, from Maguan, Yunnan; Numbers 3 and 4, from Longlin, Guangxi. The samples were prepared and tested by Xu Zhaoran at the Testing Center of Sun Yatsen University.

ature of 4000 to 6500°C usually have evergreen broad-leaf forests. The former region is considered tropical, and the latter subtropical (Wu, 1980). According to Table 3, limestone areas in southern and southwestern China extend to both tropical and subtropical zones. Forests occurring on limestone substrates in the studied areas are a mixture of evergreen and deciduous broad-leaf forests, different from the regional evergreen forests described by Wu (1980). Details on the vegetation are presented in the following section.

Forest Vegetation

The limestone vegetation of southern and southwestern China can be divided into regions as diagrammed in Figure 2. Areas of particular botanical interest are southwestern Guangxi (I), southern Yunnan (II), Nanpan Jiang area (III), southeastern Yunnan (IV), southern Guizhou and northern Guangxi (V), and western Guangxi (VI). Central Guangxi (VII) and central Guizhou (VIII) are relatively developed areas without any remaining primary forests.

The solid line in Figure 2, which separates regions I and II from the others, is similar to the boundary of tropical and subtropical climatic zones. From the dotted line northward, the annual ≥ 10°C accumulated temperature (see Table 3) is generally less than 5000°C, and from this line southward, including regions III, IV, VI, and VII, the annual ≥10°C accumulated temperature is ca. 5000–6500°C, corresponding to south-subtropical climates. Thus, we may call the forest vegetation in regions I and II "tropical limestone forests," those in III, IV, VI, and VII "south-subtropical limestone forests," and those in V and VIII "middle-subtropical limestone forests." Regardless of what they are termed, the limestone forests are different from the non-limestone forests in the same regions in both their community structure and floristic composition. In regions I and II, trees may reach 70 m in height, and some trees of Dipterocarpaceae have been recorded as 70 m tall and 2 m diameter in both southwestern Guangxi and southern Yunnan. In III, IV, VI, and VII, the forest canopy may be as high as 30–40 m, but it is usually less than 25 m in V and VIII. In all these regions, deciduous trees form an important part of the upper layer of the forest, but in the middle layer there are more evergreen trees than deciduous ones.

The vegetation in the regions east of the vertical dashed line in Figure 2 differs from that west of the line. Floristic analysis of the collections from different sites indicates that many endemic species that occur in region IV are not found west of this region, while many endemic species recorded in

←

FIGURE 1. Distribution of carbonate substrates and typical karst area (upper left corner) in China. In the main map, capital cities of the provinces of Guangxi, Guizhou, and Yunnan are represented by solid circles (Nanning, 22°50'N, 108°20'E; Guiyang, 26°34'N, 106°43'E; Kunming, 25°03'N, 102°43'E); in the insert (upper left), the corresponding cities are identified by name. Legends (bottom left): I, pure carbonate substrates. II, substrates of carbonate including small portion of noncarbonate. III, substrates of mixed carbonate and noncarbonate in similar portions. IV, substrates of noncarbonate including a small portion of carbonate. Translated and modified from the following two sources: Ren Meie & Liu Zhenzhong (1983): Outline of Karstology. Commercial Press, Beijing; and Academia Sinica (1980): Physiography of China, Topography. Science Press, Beijing.

Table 3. Some climate data of Southern and Southwestern China.*

Locality	Annual precipitation mm	Annual ≥10°C accumulated temperature °C	Yearly temperature °C	Monthly temperature Lowest °C	Monthly temperature Highest °C	Yearly humidity %	Altitude m
Hekou	1777	8249	22.6	15.3	27.5	85	137
Funing	1184	6401	19.3	10.9	25.2	79	>1000
Ningming	1350	7600	22.0	13.0	28.0	—	150
Libo	1300	4843	16.1	6.2	24.2	82	800

* Hekou, southern Yunnan; Funing, southeastern Yunnan; Ningming, southwestern Guangxi; and Libo, southern Guizhou. The annual ≥10°C accumulated temperature is the total temperature compounded from all the daily temperatures in one year that are higher than or equal to 10° Celsius (also see discussion in the text).

Xishuangbanna of southern Yunnan (Zhu, 1987) or region II are not present in the regions east of it, including regions I, IV, III and V (Chen, 1986; Liao, 1986; Liang et al., 1981, 1985; Xu, 1984, 1987).

Following are some characteristic species from the limestone vegetation in the above regions:

Southwestern Guangxi (I): *Amesiodendron chinense* (Merr.) Hu, *Cephalomappa sinensis* (Chun & How) Kosterm., *Cleistanthus saichikii* Merr., *Drypetes pereticulata* Gagnep., *Excentrodendron hsienmu* (Chun & How) H. T. Chang & R. H. Miao, *Garcinia paucinervis* Chun & How, *Parashorea chinensis* var. *kwangsiensis* Lin Chi, *Teonongia tonkinensis* Stapf, and *Walsura robusta* Roxb. Species of golden-flowered *Camellia* sect. *Chrysantha*, the so-called "giant pandas of the plant kingdom," are mostly found in this region.

Southern Yunnan (II): *Cleistanthus saichikii*, *Dracaena cochinchinensis* (Lour.) S. C. Chen, *Duabanga grandiflora* (Roxb.) Walp., *Horsfieldia* spp., *Parashorea chinensis* Wang Hsie var. *chinensis*, *Tetrameles nudiflora* R. Br., and some species of Combretaceae, Leguminosae, Meliaceae, Sapindaceae, and Sapotaceae that are often found in tropical rainforests.

Nanpan Jiang Area (III): *Carpinus* spp., *Celtis bodinieri* Lévl., *Chukrasia tabularis* var. *velutina* (Wall.) King, *Cleidiocarpon cavaleriei* (Lévl.) Airy Shaw, *Combretum alfredii* Hance, *Hiptage benghalensis* (L.) Kurz, *Koelreuteria bipinnata* Franch., *Pittosporum glabratum* Lindl., *Platycarya longipes* Y. C. Wu, *Quercus glauca* Thunb., *Sorbus purpurea* Z. R. Xu, and *Ulmus* spp. Region III is in the border area of the provinces of Guangxi, Guizhou, and Yunnan, and along the valley of Nanpan Jiang (River), which is the upper part of the Pearl River. It is interesting that even *Bombax ceiba* L., a tropical tree that is abundant in Vietnam, can be found here in some river valleys. The flora is a combination of eastern and western Chinese floristic elements.

Southeastern Yunnan (IV): *Burretiodendron esquirolii* (Lévl.) Rehd., *Carpinus pubescens* Burk., *Castanopsis fargesii* Franch., *Michelia fulva* Chang & B. L. Chen, *Miliusa chunii* W. T. Wang, *Pittosporum kerrii* Craib, *P. tonkinense* Gagnep., *Quercus glaucoides* (Schott) Rehd., and *Rapanea neriifolia* (Sieb. & Zucc.) Mez. Some species from regions I and III also appear in this region. In addition, there are some genera, such as *Calcareoboea*, *Malania*, and *Parepigynum*, that are endemic to southeastern Yunnan. This region is well known by its rich endemic and relict elements (Wu & Wang, 1985); it is especially rich in species of Magnoliaceae (Chen & Xu, 1993). I did not explore much of the neighboring western Guangxi (VI), because the limestone forests there are similar in vegetational and floristic composition to those in southeastern Yunnan; they differ in that fewer of them survive. In recent years, Wang (1990) recorded more than a hundred new species of Gesneriaceae in China, the majority of which were collected from one or a few limestone hills in western Guangxi. These species may indicate some distinctiveness between the floras of western Guangxi and southeastern Yunnan.

Southern Guizhou and northern Guangxi (V): *Acer sycopseoides* Chun, *Boniodendron minus* (Hemsl.) T. Chen, *Carpinus pubescens* Burk., *C. rupestris* A. Camus, *Clausena dunniana* Lévl., *Elaeagnus calcarea* Z. R. Xu, *Handeliodendron*

→

FIGURE 2. Regionalization of major limestone vegetations in China: I, Southwestern Guangxi; II, Southern Yunnan; III, Nanpan Jiang area; IV, Southeastern Yunnan; V, Southern Guizhou and Northern Guangxi; VI, Western Guangxi; VII, Central Guangxi; VIII, Central Guizhou.

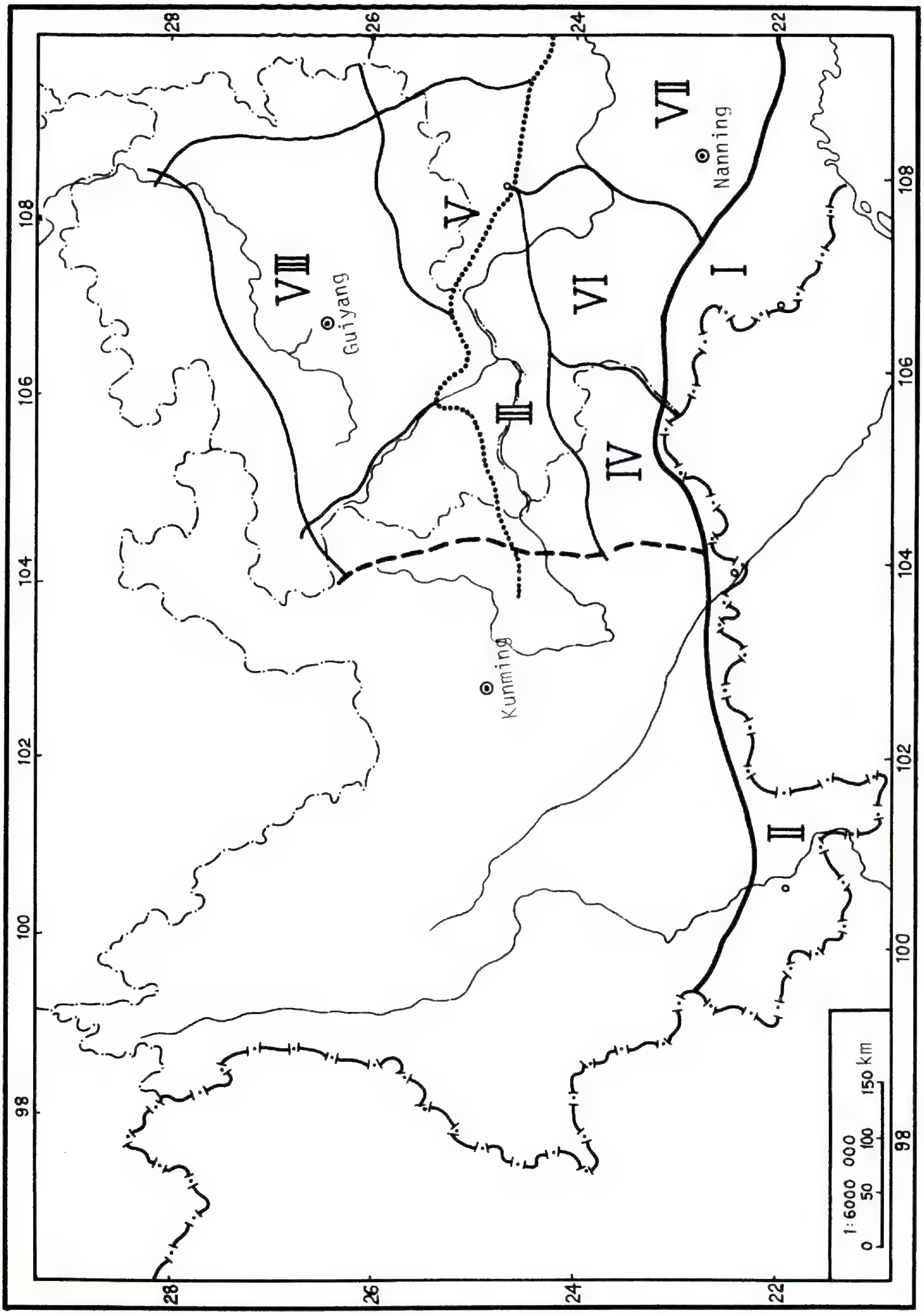
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Table 4. Size of the limestone flora of Southern and Southwestern China (Xu, 1993).

Plant group	Families	Genera	Species	Subspecies	Varieties	Forms
Pteridophyta	29	68	165	0	3	0
Gymnospermae	9	18	33	0	2	0
Angiospermae	157	1127	3778	11	271	24
(Dicotyledons)	(136)	(938)	(3263)	(11)	(239)	(22)
(Monocotyledons)	(21)	(189)	(515)	(0)	(32)	(2)
			3976	11	276	24
Total	195	1213	4287			

bodinieri (Lévl.) Rehd., *Keteleeria calcarea* Cheng & L. K. Fu, *Manglietia calcarea* X. H. Song, *Michelia angustioblonga* Law & Wu, *Pistacia chinensis* Bunge, *Pittosporum ovoideum* Gowda, *P. tetraspermum* Wight & Arn., *Platycarya longipes* Y. C. Wu, *Photinia tushanensis* Yu, *P. lochengensis* Yu, and *Pseudotsuga brevifolia* Cheng & L. K. Fu. Many of these species are endemic to southern Guizhou and northern Guangxi, where virgin forests on limestone hills have been found. A national limestone forest reserve was established in 1988 in southern Guizhou to protect the primary forests and wildlife. This reserve, where complete forest cover on the limestone hills extends for tens of kilometers, presents a rare case among the surviving limestone forests in southern and southwestern China. More than 50 new plant species have been reported from there in recent years (Xu, 1984, 1987; Zhou, 1987).

Fang & Liu (1984) claimed to have discovered an evergreen limestone forest in subtropical China (southern Sichuan). However, there are flaws in their report: (1) the forest is not on limestone hills or outcrops but on deep layers of soil from Quaternary deposits with a few limestone outcrops; and (2) the soil contains very low concentrations of calcium carbonate ($CaCO_3$), unlike that of limestone forests. In my years of extensive exploration throughout southern and southwestern China, I have found no evergreen forests on limestone outcrops or karst hills. Palynological studies of southern Guizhou limestone substrates provide evidence that the present limestone forest there is similar in floristic composition to the Tertiary flora of the same region (Zhou, 1987), suggesting that it is a primary forest. Our vegetation studies have shown that this primary forest is of mixed evergreen and deciduous trees (Xu & Sun, 1984).

Based on my sample studies, the limestone forests in southern and southwestern China are stable in population structure and differ from the acid-soil evergreen forests in the same region by: (1) having different dominant species, and the dominant species being less distinct; (2) possessing limestone-characteristic species (see Floral Features) of both evergreen and deciduous trees, especially in the upper layer of the forest; and (3) containing some tropical elements as well as some temperate elements that are seldom found in acid-soil forests in the same climatic zone or similar altitude.

It is important to point out that the limestone forests in southern and southwestern China have little similarity to arid floras, such as desert floras in tropical or subtropical areas in other parts of the world. As Table 3 shows, the region has relatively high precipitation. The degree of drought in this area is limited, and since aboveground streams can exist in limestone forests, aquatic or wetland plants can be found in this flora.

Floral Features

According to the checklist of limestone species (Xu, 1993), 4287 specific and infraspecific taxa of vascular plants have been recorded from the limestone forests in southern and southwestern China. They belong to 1213 genera and 195 families. Table 4 summarizes the size of the entire limestone flora of southern and southwestern China. Among the 195 families, 21 possess more than 50 species, which together account for 597 genera (49% of the entire flora) and 2111 species (53%) (Table 5). In the flora of China, these 21 families are also the largest families (Wu & Wang, 1985).

Following Wu (1981) and Wu & Wang (1985), the genera of spermatophytes of the limestone flora of southern and southwestern China are treated in 14 distribution types (Table 6). Compared with the flora of China as a whole (Wu & Wang, 1985), the limestone flora possesses a higher percentage of various tropical elements, including pantropical, disjunct in tropical Asia and tropical America, Old World tropic, tropical Asia to Australia, tropical Asia to tropical Africa, and tropical Asia (in total, 69.1% vs. 51.0%). Among the various tropical elements, the tropical Asian have the highest percentage (27.7%), which is also true in the Chinese flora (18.8%). This

Table 5. Families with more than 50 species in the limestone flora of Southern and Southwestern China. The arrangement of families is in order of the number of species.

Family	Gen.: Spp.	Family	Gen.: Spp.	Family	Gen.: Spp.
Leguminosae	52:224	Lauraceae	16:119	Acanthaceae	38:68
Labiatae	29:144	Rosaceae	23:112	Apocynaceae	28:63
Euphorbiaceae	50:142	Orchidaceae	38:106	Rutaceae	15:62
Rubiaceae	42:135	Liliaceae	17:100	Verbenaceae	10:61
Gesneriaceae	36:128	Moraceae	15:90	Vitaceae	7:57
Compositae	47:122	Gramineae	59:76	Araceae	17:56
Urticaceae	16:121	Asclepiadaceae	28:72	Annonaceae	14:53

result reflects the fact that the limestone area is located in the southern part of China within the tropical and subtropical zones, while the Chinese flora covers a large temperate area.

Although the limestone area is within the tropical and subtropical zones, the limestone flora includes 19.8% northern temperate and East Asian elements, a percentage comparable to that of the flora of China (20.7%). By critically examining the classification of Wu (1981), one can notice that southern and southwestern China are rich in temperate elements and are also the distribution centers of many tropical genera, such as *Annamocarya*, *Distylium*, *Eustigma*, *Camellia*, *Manglietia*, *Michelia*, *Kmeria*, and *Parakmeria*. The presence of so many temperate and tropical elements in the limestone forests in southern and southwestern China confirms that the area is rich in botanical diversity.

Since the majority of non-limestone substrates in southern and southwestern China can be roughly grouped into "acid soil," in which the soil pH appears acidic, my analysis will focus on the relationship between a limestone flora and an acid-soil flora.

Ninety-four families of vascular plants in the flora of China (IBASB, 1972–1983) have not been recorded from the limestone forests in the study area. This does not necessarily mean, however, that these 94 families occur only on non-limestone substrates in China, because limestone occurs elsewhere in China outside of the southern and southwestern portions of the country and because the

Table 6. Floristic analysis of spermatophytes of the limestone flora of Southern and Southwestern China in comparison with the entire flora of China (following Wu & Wang, 1985)

Distribution types	Limestone flora		Chinese flora	
	Genera	%	Genera	%
Cosmopolitan	49	—	108	—
Pantropical	183	16.7	372	13
Disjunct in tropial Asia and tropical America	31	2.8	89	3.1
Old World tropical	95	8.7	163	5.7
Tropical Asia to Australasia	70	6.4	150	5.2
Tropical Asia to tropical Africa	74	6.8	151	5.2
Tropical Asia	304	27.7	542	18.8
Northern temperate	108	9.9	296	10.3
Disjunct in East Asia and North America	34	3.1	117	4.1
Old World temperate	29	2.6	157	5.5
Temperate Asia	6	0.5	63	2.2
Mediterranean, West Asia to Central Asia	2	0.2	166	5.8
Central Asia	0	0	112	3.8
East Asia	108	9.9	298	10.4
Chinese endemic	52	4.7	196	6.8
Total	1145	100	2980	100 (99.9)

investigations of the limestone flora of southern and southwestern China are not complete. According to my personal observations, however, it is true that some of these families are abundant on acid soil, but never occur on the adjacent limestone outcrops, e.g., Bretschneideraceae, Clethraceae, Cyatheaceae, Gleicheniaceae, and Rhoipteleaceae. The Rhoipteleaceae are a monotypic family with a center of distribution in southern Guizhou, in which limestone outcrops are extremely common, but I have never documented any individuals of this family to occur in natural limestone habitats.

In general, large families may be distributed in more diverse habitats as well as more climate zones. But even some large families show a distinct difference in their patterns of distribution in limestone and acid soil. Ericaceae (ca. 800 spp.), Symplocaceae (80 spp.), and Theaceae (500 spp.) are three large families in the Chinese flora. Their distribution centers are actually in southern and southwestern China (Chang, 1980), but they have few representatives in the limestone flora (Ericaceae, 27 spp.; Symplocaceae, 6 spp.; Theaceae, 18 spp.). It is apparent that these families are well adapted to acid soil but not as suited to limestone. In Molan Natural Karst Reserve (southern Guizhou), I investigated the Symplocaceae and found only 2 individual plants that belonged to 2 different species in the limestone forests, while more than 20 species of this family are known to occur in acid-soil forests adjacent to the limestone reserve (Xu, 1984).

Contrary to the families that are well adapted to acid soil but not to limestone, the Chinese Gesneriaceae have a preference for limestone substrates. Among the 56 genera and 413 species of Gesneriaceae recorded in China, the majority were found on limestone outcrops or limestone hills, especially in the provinces of Guangxi, Guangdong, Guizhou, and Yunnan (Wang, 1990). Although Xu (1993) reported only 128 species of Gesneriaceae from limestone habitats, my personal collecting experience has convinced me that there are more gesneriad plants in limestone forests than in non-limestone forests. Many gesneriad species were not included in Xu (1993) because most of the available collections, other than those made by me, had no indication of habitat type; however, they probably had been collected from a limestone area. My recent monographic studies on some genera of Gesneriaceae support the theory that the family is well adapted to limestone habitats. The two gesneriad genera *Boea* and *Paraboea*, totaling more than a hundred species, are distributed from China to Southeast Asia and Australia and occur almost exclusively in limestone habitats (Xu & Burtt, 1991). Burtt (1984) and Chin (1977, 1979, 1983a, b) also recorded many Gesneriaceae species from limestone habitats in Southeast Asia. Besides Gesneriaceae, members of Euphorbiaceae, Juglandaceae, Pittosporaceae, Primulaceae, Rutaceae, Ulmaceae, and Urticaceae are apparently well adapted to limestone outcrops in southern and southwestern China.

Twenty-two genera, which are mostly monotypic or oligotypic and are confined to a small area, have been recorded as endemic to the limestone flora of southern and southwestern China, e.g., *Calcareoboea* (Gesneriaceae), *Excentrodendron* Chang & Miau (Tiliaceae), *Malania* Chun & S. Lee (Olacaceae), *Parepigynum* Tsiang & P. T. Li (Apocynaceae), and *Tengia* (Gesneriaceae). These genera represent trees, shrubs, lianas, and herbs, of which the herbal Gesneriaceae genera make up a large percentage. Most of these 22 genera have a distribution center in Guangxi, Guizhou, and Yunnan, which one might expect since the three provinces possess the majority of limestone substrates in China (Fig. 1).

There are a number of large genera characteristic of the limestone flora. Each large genus is not exclusively distributed in limestone habitats, but most of its species are endemic in the limestone flora. Examples include several Gesneriaceae genera, *Paraboea* (85 spp.), *Chirita* (130 spp.), *Hemiboea* (22 spp.).

In addition, some monotypic or oligotypic genera have been found to occur mostly in limestone habitats rather than in acid soil. Many of them have woody species that dominate the limestone forests in southern and southwestern China. Following are some examples, with the number of species recorded from the limestone forests in southern and southwestern China: *Burretiodendron* (4 spp., Tiliaceae), *Cephalomappa* (1 sp., Euphorbiaceae), *Delavaya* (1 sp., Sapindaceae), *Echinacanthus* (3 spp., Acanthaceae), *Pistacia* (2 spp., Anacardiaceae), *Platycarya* (3 spp., Juglandaceae), *Tetrameles* (1 sp., Datiscaceae), *Tetrathyrium* (1 sp., Hamamelidaceae), *Zenia* (1 sp., Leguminosae).

Contrary to the above genera characteristic of the limestone flora, some genera are characteristic of the acid-soil flora, with an abundance of species in acid-soil habitats, but few of their species occur in limestone forests within the same geographic region. These genera, though they do not represent the limestone flora, help reveal the relationship between a limestone flora and an acid-soil flora. Following are some examples; the numbers of species that have been recorded from limestone habitats versus those from acid-soil habitats in China are

listed in parentheses: *Camellia* (16:200, i.e., 16 spp. recorded in limestone area, while 200 spp. from acid soil; Theaceae), *Eurya* (0:80; Theaceae), *Hydrangea* (3:45; Saxifragaceae), *Pinus* (3:22; Pinaceae), *Rhododendron* (14:650; Ericaceae), *Symplocos* (6:77; Symplocaceae), and *Vaccinium* (8:45; Ericaceae). Although the current study focuses on southern and southwestern China, Xu (1993) had attempted to identify all of the Chinese limestone species of these genera by an exhaustive survey of the literature. The above ratio, therefore, may be taken as Chinese limestone species versus Chinese acid-soil species of the concerned genus. Within an acid-soil characteristic genus, there may be limestone characteristic sections or species. *Camellia* is a good example. Ninety percent of its species are found in acid soil, but the section of golden-flowered camellia, section *Chrysantha* Chang, is characteristic of limestone forests in southwestern Guangxi. Ten of the 15 species are exclusively distributed in limestone habitats, and the rest are exclusively distributed in acid-soil habitats, with only one exception that occurs in both acid-soil and limestone habitats (Ye et al., 1993).

Distinctions between a limestone flora and an acid-soil flora are best demonstrated at the species level. According to Chin (1977, 1979, 1983a, b), limestone species are classified into four groups in terms of their distribution patterns related to the limestone substrates: (1) *endemics*, species that are distributed exclusively in limestone habitats; this group comprises about 20% of the total number of species that have been recorded in the limestone flora in southern and southwestern China (Xu, 1993; the following percentages refer to the same source); (2) *preferents*, species that are more abundant on limestone, but rarely found on acid soil (about 10% of the species); (3) *indifferents*, species that show no distinct difference in their distribution in limestone and in acid soil; this group is the majority (about 65% of the species) in the limestone flora; (4) *strangers*, species that are more abundant in acid soil and seldom found on limestone (about 5% of the species). The endemics and preferents can be considered to be "limestone-characteristic species." More limestone-characteristic species may be found in a primary limestone forest than in a secondary one because human disturbance on a primary forest may eliminate some relict species, and, during recolonization in a limestone area, the relicts may not be able to return (Xu, 1986). These characteristic species are usually dominants in the limestone forests, regardless of whether they are trees, shrubs, or herbs that make up different layers of the forest.

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CHROMOSOMAL CYTOLOGY AND EVOLUTION IN EUPATORIEAE (ASTERACEAE)[1]

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Abstract

Reports of 68 new chromosome counts attributed to 53 species from 25 genera of Eupatorieae of the Asteraceae, based mostly on determinations of mitotic materials, include first counts for 2 genera (*Acanthostyles* and *Lepidesmia*) and 14 species and new reports for 8 species. B chromosomes are reported for 4 genera and 12 species. Karyotype analyses made on 20 species of Eupatorieae and one species of Heliantheae showed that total karyotypic lengths of the taxa with n = 16–19 of helianthoid and eupatorioid taxa are comparable to those of some eupatorioid taxa with n = 10. This is contrary to the previous hypothesis that the higher chromosome numbers n = 16–19 were derived from n = 10 by polyploidization followed by dysploid loss. Cytological data supplement and are consistent with the following conclusions predicted from molecular phylogenetical and biochemical data: (1) The ultimate base number of Eupatorieae is 17, and the lower numbers are derived by successive dysploid reductions; (2) A reduction in chromosomal and total karyotypic length accompanied by evolutionary advancement has been revealed for some genera and species within this tribe; (3) A high base number of x = 17 in Eupatorieae is considered to be derived directly from one of the members of Heliantheae with n = 17 to 19.

The Eupatorieae are a very diversified tribe and consist of more than 2300 species and 180 genera in 18 subtribes (King & Robinson, 1987). In spite of its representing a large element of Asteraceae, the tribe Eupatorieae has remained poorly understood because its centers of diversity are in Central America and South America, which were remote from the early centers of study. Recent monographical (King & Robinson, 1987), cladistical (Karis, 1993; Bremer, 1987, 1994; Bremer et al., 1992), and molecular phylogenetic works (Jansen et al., 1991; Watson et al., 1991; Kim et al., 1992) have stimulated us to study the origin and evolution of Eupatorieae. A broad range of base chromosome numbers, from n = 4 to 25, has been reported (Fig. 1). The remarkable constancy of chromosome morphology and number within genera has been invaluable in the study of plant systematics. In addition, many of the groups that have distinctive chromosome numbers are among the most readily defined taxonomically. Therefore, we attempted to find clues regarding phylogenetic relationships throughout the tribe by assessing chromosomal variation, restriction site mutations in chloroplast DNA (Ito et al., in prep.), and the utility of isozyme number for determining ploidy level (Suzuki et al., unpublished).

Our purposes in this paper are to report original counts of chromosome numbers in Eupatorieae, to compare them with numbers reported previously, to analyze karyotypes of selected representative species, and to apply the results to a better understanding of the origin, phylogeny, and chromosomal evolution in the tribe.

Materials and Methods

Cytological observations were made primarily on mitotic cells of root tips obtained from seedlings

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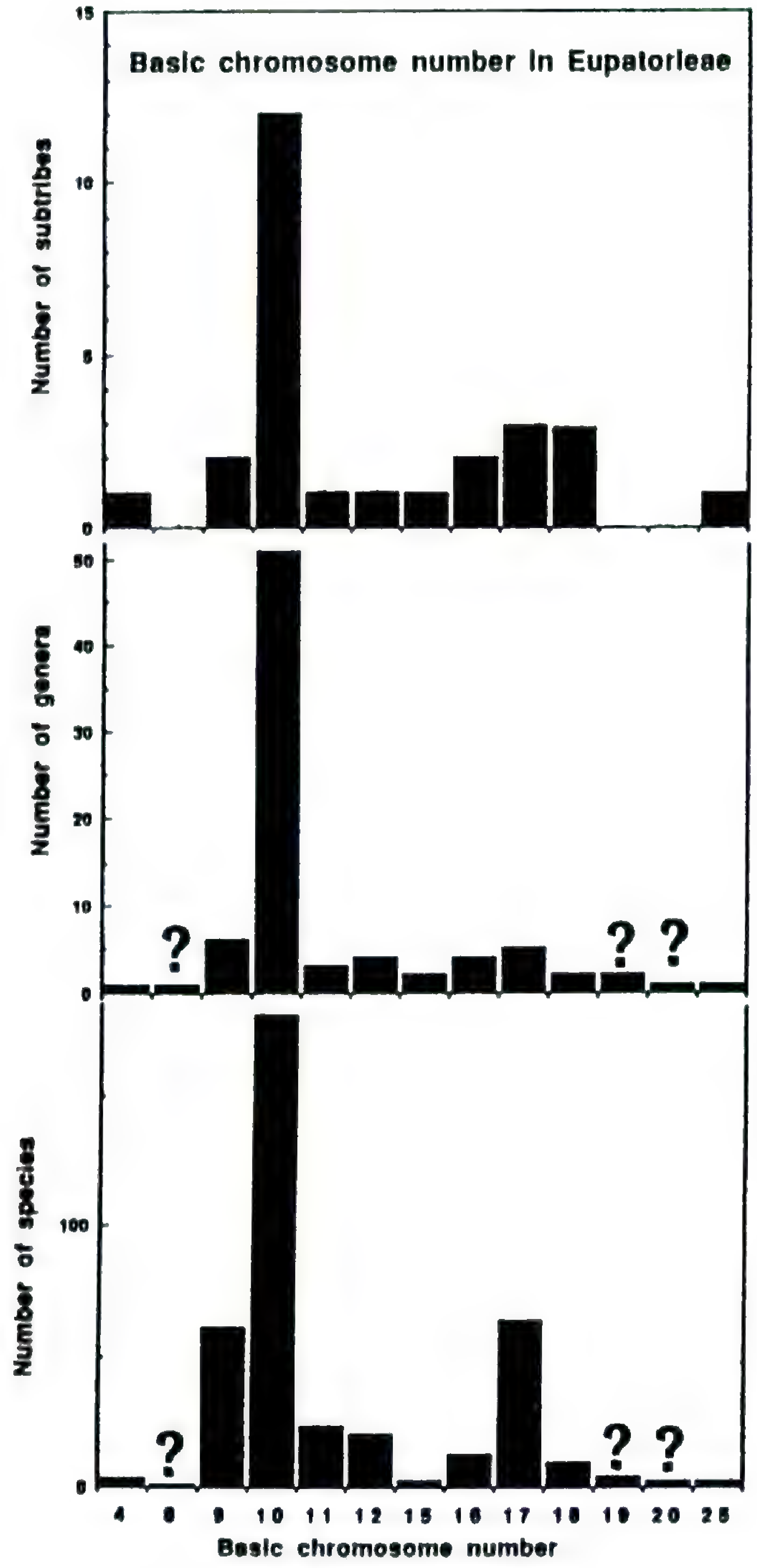


FIGURE 1. Distributions of base chromosome numbers in the species, genera, and subtribes of Eupatorieae.

grown from fruits of known provenance in the greenhouse of Kobe University. For the cultivation of specimens and the preparation of materials for cytological work, the procedures of Watanabe et al. (1975) and Watanabe et al. (1990) were followed. The measurements of karyotypic length were made on root tip mitoses, and each measurement is the mean of measurements of five metaphase plates. Voucher specimens have been deposited in US and MAK.

RESULTS

CHROMOSOME NUMBER

All chromosome number determinations are presented in Table 1. Figures 2–9 are photographs of somatic chromosomes of eight representative species.

Chromosome number determinations from 68 populations attributed to 53 species from 25 genera of Eupatorieae are reported. First reports are given for *Acanthostyles buniifolius* ($2n$ = 20), *Adenostemma cuatrecasasii* ($2n$ = 20), *Ageratina isolepis* ($2n$ = 34), *Decachaeta thieleana* (2n = 32), *Fleischmannia sideritides* (n = 10, $2n$ = 20), *Lepidesmia squarrosa* ($2n$ = 20), *Koanophyllon longifolium* ($2n$ = 30), *Mikania congesta* ($2n$ = 34+0 to 3B), *Neomirandea arthodes* (n = 17+0 to 5B), *N. guevarii* ($2n$ = 50+2 to 12B), *N. parasitica* ($2n$ = 34+1 to 2B), *N. standleyi* ($2n$ = 50+1 to 4B), *Stevia connata* ($2n$ = 44), and *S. suaveolens* ($2n$ = 33+0 to 2B), and new reports are given for *Ageratina rothrockii* ($2n$ = 51 and 68), *Chromolaena laevigata* ($2n$ = 50), *Mikania scandens* ($2n$ = 34+0 to 1B), *Praxelis clematidea* ($2n$ = 30), *Stevia eupatoria* ($2n$ = 36 and 48), *S. monardaefolia* ($2n$ = 33), *S. pilosa* ($2n$ = 22), and *S. tomentosa* ($2n$ = 33). Remaining counts confirm the chromosome numbers of all previous reports in some species or are consistent with one of variable numbers reported by several authors in other species. Of the 68 accessions in which chromosome number determinations were made, 53 (78%) are diploids and 15 (22%) are polyploids. The presence of B chromosomes is reported for *Bartlettina sordida, Mikania congesta, M. scandens, Neomirandea angularis, N. arthodes, N. biflora, N. guevarii, N. parasitica, N. standleyi, Stevia salicifolia, S. serrata,* and *S. suaveolens.* The B chromosomes are distinctly smaller than autosomes (Fig. 3), except in *Mikania* and *Stevia,* and show the early condensation typical of the centromeric regions of autosomes at prometaphase. Both *Mikania* and *Stevia* have several small autosomes nearly the same size as B chromosomes (Fig. 7).

KARYOTYPE

Figures 10–30 are haploid karyotypic idiograms. Drawings are based on the means of ten measurements for each. Each idiogram is arranged in descending order of chromosome number within Eupatorieae next to helianthoid *Podachaenium eminens* (Lag.) Sch. Bip.

In Table 2, we report the mean total karyotypic lengths with their standard errors, the ranges from the longest to shortest chromosome and their ratio for each karyotype, and the mean arm ratio (total long arm length/total short arm length). The two latter measurements indicate the degree of karyotypic asymmetry. Significance of differences be-

Table 1. Chromosome number determinations in Eupatorieae (Asteraceae). *n* counts are from pollen mother cells (PMCs) and 2*n* counts are from root tips.

Species	Chromosome number *n*	Chromosome number 2*n*	Collection data or reference
Acanthostyles buniifolius (Hook. & Arn.) R. M. King & H. Rob.	—	20	Argentina. Buenos Aires: Parana Delta at Tigre, *King 10288* (US)
Adenostemma cuatrecasasii R. M. King & H. Rob.	—	20	Venezuela. Mérida: 4.1–6.3 km E of Santo Domingo, *King 10538* (US)
A. viscosum J. R. & G. Forst.	—	20	U.S.A. Hawaii: Kawaihau Dist., Makaleha Mtns., SSW of Puu Eu, *Flynn 5461* (US)
Ageratina anisochroma (Klatt) R. M. King & H. Rob.	—	34	Costa Rica. 10 km W of San Ramon, 15 Aug. 1991, *Yahara et al. s.n.* (MAK)
A. bustamenta (DC.) R. M. King & H. Rob.	17II	—	Costa Rica. 27 km N of San Isidro, *Yahara et al. 39* (MAK)
A. havanensis R. M. King & H. Rob.	—	34	Mexico. Coahuila: near the University in Saltillo, *King 10379* (US)
A. herbacea (A. Gray) R. M. King & H. Rob.	—	34	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, *Yahara et al. 13* (MAK)
A. isolepis (B. Rob.) R. M. King & H. Rob.	—	34	Mexico. Hidalgo: 13 km from Pachuca to El Chico on Hwy. 105, 17 Oct. 1993, *Yahara & Ito s.n.* (MAK)
A. rothrockii (A. Gray) R. M. King & H. Rob.	—	51	U.S.A. Texas: Jeff Davis Co., Old Fort Davis, *Yahara et al. 7* (MAK)
	—	68	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, 9 Aug. 1991, *Yahara et al. s.n.* (MAK)
A. scorodonioides (A. Gray) R. M. King & H. Rob.	—	34	Mexico. Coahuila: 2 km E of Los Lirios, *King 10326* (US)
A. wrightii (A. Gray) R. M. King & H. Rob.	—	34	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, *Yahara et al. 14* (MAK)
Ageratum corymbosum Zuccagni	—	40	Mexico. Coahuila: 3 km S of Saltillo, *King 10368* (US)
A. microcarpum (Benth. in Orsted) Hemsl.	10II	—	Costa Rica. 15 km S of Cartago, *King 10236* (US)
	10II	—	Costa Rica. 27 km N of San Isidro, *King 10241* (US)
	10II	—	Costa Rica. Volcan Irazu, 16 Aug. 1991, *Yahara et al. s.n.* (MAK)
	10II	—	Costa Rica. 0.5 km NE from Mt. Orosi, 17 Aug. 1991, *Yahara et al. s.n.* (MAK)
	10II	20	Costa Rica. The summit of Mt. Orosi, 17 Aug. 1991, *Yahara et al. s.n.* (MAK)
Austroeupatorium inulaefolium (HBK) R. M. King & H. Rob.	—	20	Argentina. Buenos Aires: city limit of Buenos Aires at the municipal train station, *King 10282* (US)
Bartlettina sordida (Less.) R. M. King & H. Rob.	16II	—	Australia. Victoria: cultivated in Royal Botanic Gardens, *King s.n.* (US)
	—	32 + 1B	Australia. Victoria: cultivated in Royal Botanic Gardens, *Watanabe s.n.* (MAK)
Brickellia betonicifolia A. Gray	—	18	U.S.A. Texas: Jeff Davis Co., Old Fort Davis, *Yahara et al. 8* (MAK)
B. chlorolepis (Wooten & Standl.) Shinners	—	18	Mexico. Coahuila: ca 9 km from Los Lirios, *King 10320* (US)
B. grandiflora (Hook.) Nutt.	—	18	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, 9 Aug. 1991, *Yahara et al.* (MAK)
Carminatia tenuiflora DC.	—	20	U.S.A. Texas: Jeff Davis Co., Davis Mts., *Yahara et al. 4* (MAK)

Table 1. Continued.

Species	Chromosome number n	Chromosome number $2n$	Collection data or reference
Carphochaete bigelovii A. Gray	—	22	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, 9 Aug. 1991, *Yahara et al. s.n.* (MAK)
Chromolaena laevigata (Lam.) R. M. King & H. Rob.	—	50	Argentina. Buenos Aires: Parana Delta at Tigre, *King 10287* (US)
Conoclinium gregii (A. Gray) Small	—	20	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, *Yahara et al. 15* (MAK)
Decachaeta thieleana (Klatt. ex T. Durand & Pittier) R. M. King & H. Rob.	—	32	Costa Rica. San José: San Ramon de Tres Rios, Nov. 1993, *Castro s.n.* (US)
Fleischmannia microstemon (Cass.) R. M. King & H. Rob.	4II	—	Costa Rica. 3 km S of Santa Cruz, *King 10243* (US)
	4II	—	Costa Rica. 0.5 km NE and on the foot of Mt. Orosi, 17 Aug. 1991, *Yahara et al. s.n.* (MAK)
F. sideritides (Benth. in Orsted) R. M. King & H. Rob.	10II	20	Costa Rica. Tapanti National Wildlife Refuge, *Yahara et al. 128* (MAK) & *King 10262* (US)
Hebeclinium macrophyllum (L.) DC.	—	20	Trinidad & Tobago. St. George: along North Coast Road, 5 km W of Maracas Bay Village, *Wasshausen 1811* (US)
Isocarpha atriplicifolia (L.) R. Br. ex DC.	—	20	Costa Rica. Santa Rosa N. P., 15 Aug. 1991, *Yahara et al. s.n.* (MAK)
Koanophyllon longifolium (B. Robinson) R. M. King & H. Rob.	—	30	Mexico. Coahuila/Nuevo Leon: just E of Laguna de Sanchez, *King 10341* (US)
Lepidesmia squarrosa Klatt.	—	20	Venezuela. Falcon: ca. 80 km S of Coro, *King 10270* (US)
Liatris spicata (L.) Willd.	—	20	U.S.A. North Carolina: Spring Lake, 7 Oct. 1991, *Ito et al. s.n.* (MAK)
Microspermum debile Benth.	—	24	Mexico. Oaxaca: 47.7 km from Guelatao, 30 Sep. 1993, *Yahara & Ito 231* (MAK)
var. *arsenei* Benth.	—	24	Mexico. Michoacán: near Patzcuaro, *Medina 2610* (US)
Mikania congesta DC.	—	34 + 0 ~ 3B	Argentina. Buenos Aires: city of Buenos Aires, banks of the Rio de La Plata, *King 10285* (US)
M. scandens L. Willd	—	34 + 0 ~ 1B	U.S.A. North Carolina: Spring Lake, 6 Oct. 1991, *Ito et al. s.n.* (TI)
Neomirandea angularis (B. L. Rob.) R. M. King & H. Rob.	—	50	Costa Rica. Cartago: Volcan Turrialba, *King 9695* (US)
	—	50 + 1B	Costa Rica. San José: La Lucha, 27 July 1987, *Lellinger s.n.* (US)
	25II	—	Costa Rica. 15 km S of Cartago, *Yahara et al. 22* (MAK) & *King 10238* (US)
	25II	—	Costa Rica. 0.5 km NE from Mt. Orosi, *Yahara et al. 66* (MAK) & *King 10244* (US)
	25II	—	Costa Rica. Near the summit of Mt. Orosi, *King 10246* (US)
N. arthodes (B. L. Rob.) R. M. King & H. Rob.	17II + 0 ~ 5B	—	Costa Rica. Cartago to San Isidro, *King 10239* (US)
	17II + 0 ~ 2B	—	Costa Rica. Cartago: Tapanti National Wildlife Refuge, *King 10261* (US)
N. biflora R. M. King & H. Rob.	—	50 + 2 ~ 6B	Costa Rica. Volcan Turrialba, *Yahara et al. 90* (MAK) & *King 10251* (US)
N. guevarii R. M. King & H. Rob.	—	50 + 12B	Costa Rica. Cartago: ca. 7 km from the dam at Tapanti, *King 9674* (US)

Table 1. Continued.

Species	Chromosome number: n	Chromosome number: 2n	Collection data or reference
	—	50 + 2 ~ 6B	Costa Rica. Cartago: Tapanti National wildlife Refuge, *King 10260* (US)
N. parasitica (Klatt) R. M. King & H. Rob.	—	34 + 1 ~ 2B	Costa Rica. 8.9 mi. NW of centers of Sanramin, *Croat 68077* (US)
N. standleyi (B. L. Rob.) R. M. King & H. Rob.	—	50 + 1 ~ 4B	Costa Rica. La Hondura, Bruallio Carrillo N. P., *King 10263* (US)
Polyanthina nemorosa (Klatt) R. M. King & H. Rob.	10II	—	Costa Rica. On the way from Cartago to Tapanti, W limit of Paraiso, *King 10247* (US)
Praxelis clematidea (Griseb.) R. M. King & H. Rob.	—	30	Brazil. Rio de Janeiro: City limit of Rio de Janeiro, *King 10281* (US)
Sclerolepis uniflora (Walter) Britten, Sterns & Poggenb.	—	30	U.S.A. Delaware: Sussex Co. W of Bethamy Beach, *King 10235* (US)
Stevia connata Lag.	—	44	Mexico. Oaxaca: 4.9 km from El Punto. *Yahara & Ito 142* (MAK)
S. eupatoria (Spreng.) Willd.	—	36	Mexico. 80 km from Mexico City to Tulancingo, *Yahara & Oyama 414* (MAK)
	—	48	Mexico. Hidalgo: 11 km from entrance to El Chico, *Yahara & Oyama 451* (MAK)
S. microchaeta Sch. Bip.	—	24	Mexico. Oaxaca: 54.3 km N from Guelatao, *Yahara & Ito 251* (MAK)
S. monardaefolia HBK	—	33	Mexico. Puebla: 4 km N from State border, Ixta-Popo N. P., *Yahara & Ito 381* (MAK)
S. ovata Willd.	—	33	U.S.A. Texas: Brewster Co., Chisos Mts. Mt. Emory, 9 Aug. 1991, *Yahara et al. s.n.* (MAK)
S. pilosa Lag.	—	22	Mexico. Hidalgo: 10 km from Pachuca to El Chico on Hwy. 105, *Yahara & Ito 425* (MAK)
S. salicifolia Cav.	—	24 + 0 ~ 1B	Mexico. Coahuila: 2 km E of Los Lirios, *King 10327* (US)
S. serrata Cav.	—	33 + 0 ~ 2B	Mexico. 80 km from Mexico City to Tulancingo, *Yahara & Oyama 412* (MAK)
S. suaveolens Lag.	—	33 + 0 ~ 2B	Mexico. 80 km from Mexico City to Tulancingo, 16 Oct. 1993, *Yahara & Oyama s.n.* (MAK)
S. tomentosa HBK	—	33	Mexico. 80 km from Mexico City to Tulancingo, *Yahara & Oyama 411* (MAK)
	—	33	Mexico. Hidalgo: 10 km from Pachuca to El Chico on Hwy. 105, *Yahara & Oyama 422* (MAK)

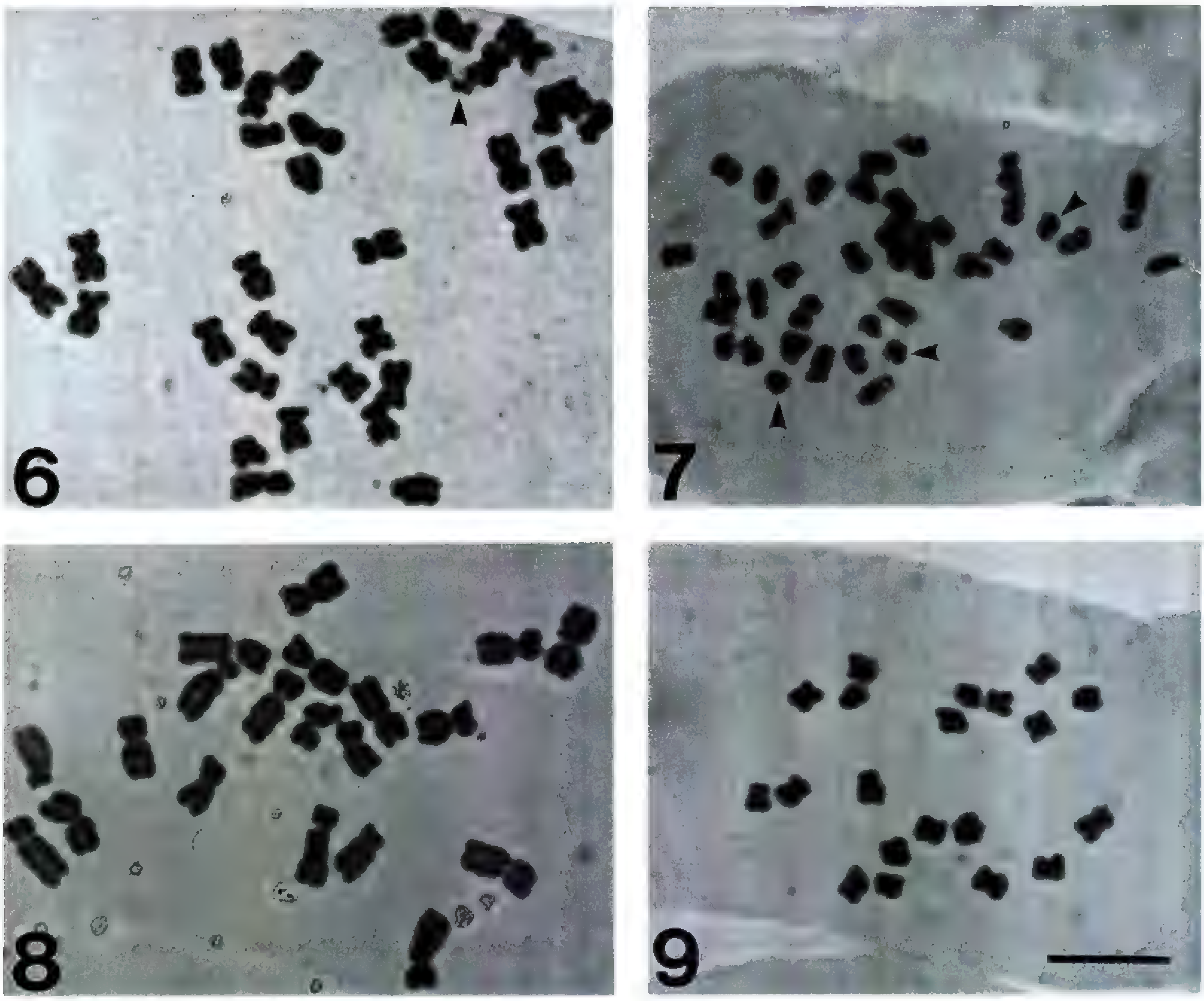
tween mean total karyotypic lengths were evaluated by a t-test.

Although there is some variation in the total karyotypic lengths and chromosomal lengths between and within species, there is a significant length difference between genera within Eupatorieae. The chromosomal and total karyotypic length of *Mikania congesta* have been shown to be significantly shorter than those of *Neomirandea parasitica* ($t = 11.137$; $p < 0.001$), *Ageratina scorodonioides* ($t = 10.943$; $p < 0.001$), and *A. havanensis* ($t = 9.976$; $p < 0.001$) with the same chromosome number, $2n = 34$ (Table 2 and Figs. 4–7). Similarly, those of *Koanophyllon longifolium* ($t = 5.027$; $p < 0.01$), *Lepidesmia squarrosa* ($t = 3.851$; $p < 0.02$), and *Fleischmannia sideritides* ($t = 5.868$; $p < 0.005$) have also been shown to be significantly shorter (compared with the total karyotypic length in *Hebeclinium macrophyllum*) than those of other species and genera with the same chromosome number, $2n = 20$ (Figs. 8, 9). The chromosomal and total karyotypic lengths of *Carphochaete bige-*

FIGURES 2–5. Photomicrographs of somatic metaphase chromosomes.—2. *Podachaenium eminens* ($2n = 38$).—3. *Neomirandea guevarii* ($2n = 50+12B$).—4. *Ageratina scorodonioides* ($2n = 34$).—5. *Bartlettina sordida* ($2n = 32+1B$). Scale bar = 5 μm. Arrow heads indicate B chromosomes.

lovii with $2n = 22$ are the longest among the taxa examined in this tribe.

Total karyotypic length of *Podachaenium eminens* ($2n = 38$, Heliantheae) is comparable to that of *Ageratina scorodonioides* ($t = 2.189$; $0.10 > p > 0.05$) with $2n = 34$, as well as to that of *Bartlettina sordida* with $2n = 32$ ($t = 0.374$; $p > 0.50$). In addition, total karyotypic lengths of those spe-



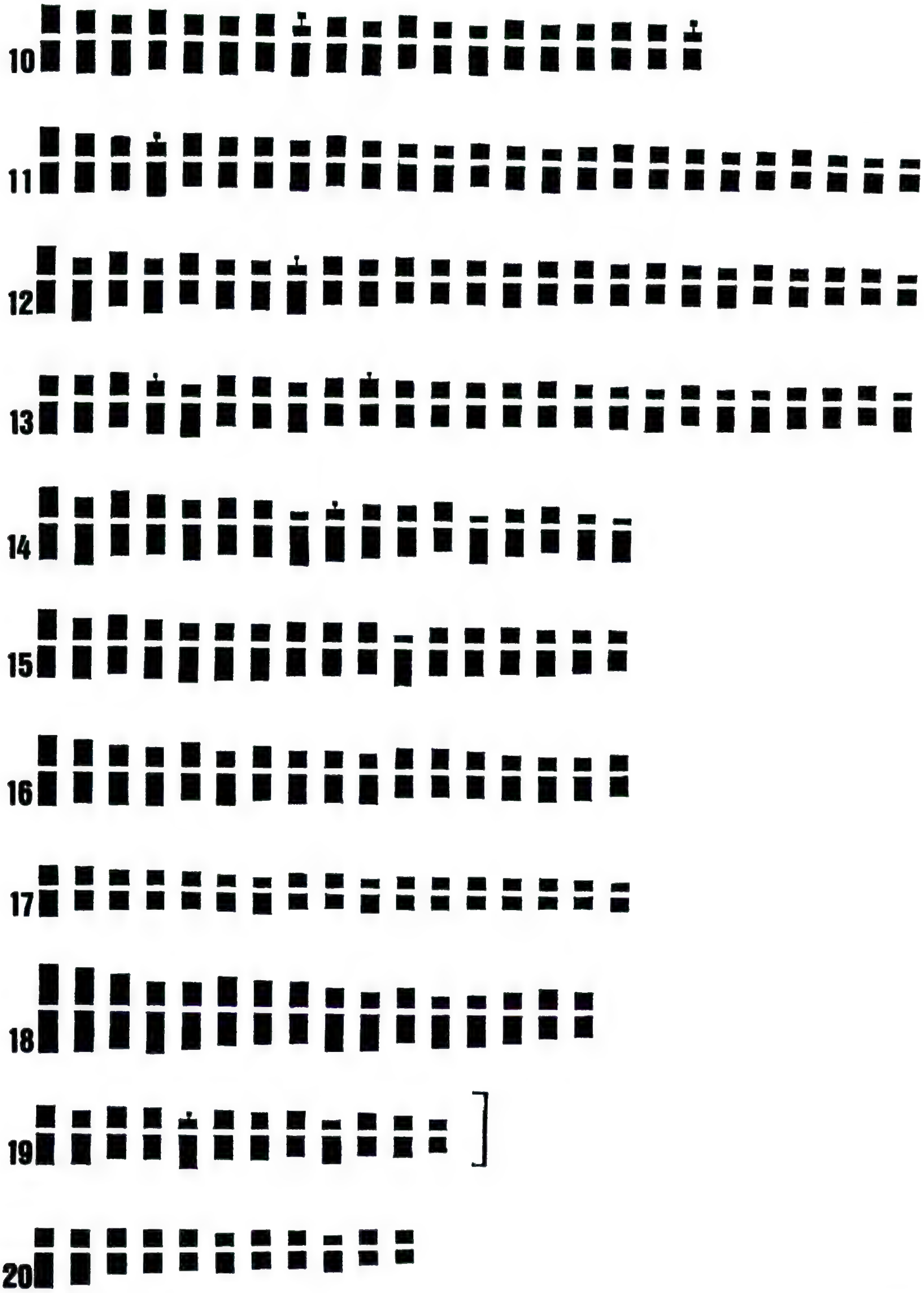
FIGURES 6–9. Photomicrographs of somatic metaphase chromosomes.—6. *Neomirandea parasitica* ($2n = 34+1B$).—7. *Mikania congesta* ($2n = 34+3B$).—8. *Eupatorium purpureum* ($2n = 20$).—9. *Fleischmannia sideritides* ($2n = 20$). Scale bar = 5 μm. Arrow heads indicate B chromosomes.

cies with higher chromosome numbers are also comparable (compared with the total karyotypic length in *Ageratina havanensis*) to those of *Austroeupatorium inulaefolium* (t = 1.212; $0.40 > p > 0.20$) and *Acanthostyles buniifolius* (t = 1.703; $0.20 > p > 0.10$), with $2n = 20$. In contrast, the difference between the total karyotypic length of species with $2n = 50$ and 34 within the genus *Neomirandea* is significant (t = 4.399; $p < 0.02$).

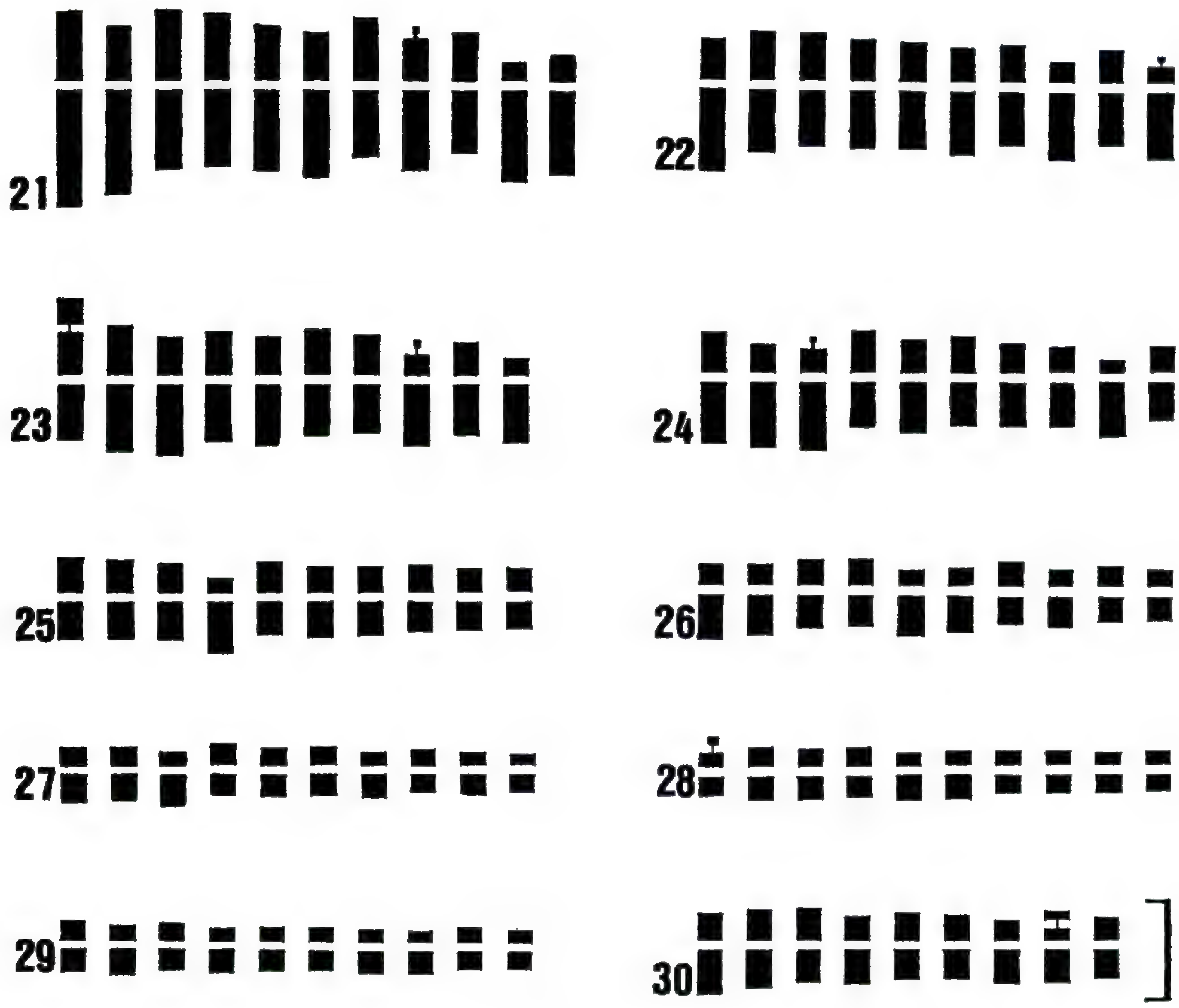
Discussion

Although previous reports for chromosome numbers have included many miscounts or have been based on misidentified plant materials, chromosome numbers, including the present data, are now known for over 467 species in 96 genera of all 18 subtribes of Eupatorieae (compiled from the indexes to chromosome numbers for 1958–1991: Fedorov, 1974; Goldblatt, 1981, 1984, 1985, 1988; Goldblatt & Johnson, 1990, 1991). These represent over 20% of the species and 53% of the genera in the tribe. Excluding ambiguous and erratic counts from previous reports, Table 3 shows the revised distribution of base chromosome numbers in Eupatorieae according to the subtribes of King & Robinson (1987); underlined base chromosome numbers were confirmed in the present study. We have not yet determined chromosome numbers for Hofmeisteriinae and Oaxacaniinae ourselves, and we are not certain if the base number $x = 18$ is correct.

The overall distribution of chromosome numbers in Eupatorieae is characterized by having few species and genera with numbers smaller than $n = 10$, compared to all other tribes in Asteraceae except Barnadesieae, Mutisieae, and Cynareae. Also, Eupatorieae is unusual because the lower chromosome numbers are completely lacking in six of the subtribes, and the occurrences of B chromosomes are confined to the groups with higher chromosome numbers. In groups with lower chromosome numbers (n = 4, 9, and 10), we have not found any B chromosomes, although we have examined the chromosomes of more than 2000 individuals (Wa-



FIGURES 10–20. Haploid karyotypic idiograms.—10. *Podachaenium eminens* (*n* = 19).—11. *Neomirandea biflora* (*n* = 25).—12. *Neomirandea guevarii* (*n* = 25).—13. *Neomirandea angularis* (*n* = 25).—14. *Ageratina scorodonioides* (*n* = 17).—15. *Neomirandea parasitica* (*n* = 17).—16. *Ageratina havanensis* (*n* = 17).—17. *Mikania congesta* (*n* = 17).—18. *Bartlettina sordida* (*n* = 16).—19. *Stevia salicifolia* (*n* = 12).—20. *Stevia pilosa* (*n* = 11). Scale bar = 4 μm.



FIGURES 21–30. Haploid karyotypic idiograms.—21. *Carphochaete bigelovii* (n = 11).—22. *Austroeupatorium inulaefolium* (n = 10).—23. *Acanthostyles buniifolius* (n = 10).—24. *Eupatorium purpureum* (n = 10).—25. *Adenostemma cuatrecasasii* (n = 10).—26. *Hebeclinium macrophyllum* (n = 10).—27. *Koanophyllon longifolium* (n = 10).—28. *Lepidesmia squarrosa* (n = 10).—9. *Fleischmannia sideritides* (n = 10).—30. *Brickellia chlorolepis* (n = 9). Scale bar = 4 μm.

tanabe, 1986; Watanabe et al. 1990; the present and unpublished data).

In the genus *Neomirandea*, all of the species examined have a variable number of B chromosomes between individuals. It is uncertain at present what role B chromosomes play in chromosomal evolution in the groups with higher chromosome numbers.

To obtain an accurate picture of the nature of higher base numbers in Eupatorieae, we have studied the genus *Neomirandea* more intensely. In previous studies, a wide range of chromosome numbers, from n = 14 to 26, has been reported, and it has been uncertain what the true base chromosome number is for this genus. The six species examined have a chromosome number of either $2n$ = 34 or 50. Previously reported chromosome numbers are also around n = 17 or n = 25, except for n = 14 in *N. ovadensis* R. M. King & H. Rob. (Strother, 1983) and n = 20 in *N. burgeri* R. M. King & H. Rob. (King et al., 1976). Thus, two different lineages based on dibase chromosome numbers have been revealed within this genus. The subdivision of *Neomirandea* based on chromosome number is supported by many synapomorphic mutations in chloroplast DNA (Yahara et al., in prep.).

Dibase chromosome numbers of 4 and 10, and of 11 and 12, are also confirmed in *Fleischmannia* and *Stevia*. Although there are many reports of $2n$ = 34, suggesting the occurrence of x = 17 in *Stevia*, those reports appear to be miscounts.

In the genus *Ageratina*, we examined eight species. *Ageratina rothrockii* has two cytotypes $3x$ and $4x$ based on x = 17. *Ageratum corymbosum* ($2n$ = $4x$ = 40), *Chromolaena laevigata* ($2n$ = $5x$ = 50), *Koanophyllon longifolium* ($2n$ = $3x$ = 30), and *Praxelis clematidea* ($2n$ = $3x$ = 30) are polyploids based on x = 10.

The distribution of base chromosome numbers in the species, genera, and subtribes is shown in Figure 1. Among them, the base numbers of x = 8, 19, and 20 reported in a few species and genera appear to be miscounts. Although there is a small

Table 2. Karyotype data for the helianthoid species *Podachaenium eminens* and 20 representative eupatorioid species. *: B chromosomes are excluded from these measurements. **: Because *Koanophyllon longifolium* is regarded as an autotriploid, its total karyotypic length is calculated as two-thirds of total genome.

Species	Chromosome number (2*n*)	Total karyotypic length (μm) mean ± S.E.	Range longest–shortest (ratio)	Mean arm ratio long arms/short arms
Podachaenium eminens	38	96.22 ± 2.25	3.21–1.91 (1.68)	1.47
*Neomirandea biflora**	50	107.06 ± 4.02	3.30–1.40 (2.36)	1.51
*N. guevarii**	50	103.60 ± 2.93	3.24–1.20 (2.70)	1.48
*N. angularis**	50	102.22 ± 3.23	2.73–1.62 (1.69)	1.52
*N. parasitica**	34	83.84 ± 1.87	3.30–1.81 (1.82)	1.71
Ageratina scorodonioides	34	88.20 ± 2.38	3.57–1.96 (1.82)	1.90
A. havanensis	34	83.48 ± 2.20	3.37–1.87 (1.80)	1.38
*Mikania congesta**	34	52.62 ± 1.67	2.40–1.14 (2.11)	1.31
*Bartlettina sordida**	32	97.62 ± 2.48	4.51–2.05 (2.20)	1.41
Stevia salicifolia	24	53.24 ± 1.63	2.82–1.60 (1.76)	1.52
S. pilosa	22	42.50 ± 0.60	2.87–1.43 (2.01)	1.40
Carphochaete bigelovii	22	117.14 ± 4.01	7.12–4.43 (1.57)	1.70
Austroeupatorium inulaefolium	20	77.14 ± 4.13	4.89–3.27 (1.50)	1.79
Acanthostyles buniifolius	20	76.98 ± 2.61	4.83–2.96 (1.63)	1.56
Eupatorium purpureum	20	63.78 ± 3.06	3.98–2.53 (1.57)	1.74
Adenostemma cuatrecasasii	20	47.78 ± 0.76	2.82–1.92 (1.47)	1.34
Hebeclinium macrophyllum	20	42.60 ± 2.15	2.54–1.64 (1.55)	1.63
Lepidesmia squarrosa	20	30.98 ± 1.63	1.68–1.16 (1.45)	1.32
*Koanophyllon longifolium***	30	29.46 ± 0.91	1.72–1.14 (1.51)	1.49
Fleischmannia sideritides	20	27.68 ± 0.73	1.59–1.17 (1.36)	1.42
Brickellia chlorolepis	18	42.66 ± 1.33	2.87–2.07 (1.39)	1.31

peak at x = 17, 12 of the 18 subtribes, 73% of the reported genera, and 53% of the reported species have chromosome numbers based on x = 10. Thus, the predominant chromosome number among species, genera, and subtribes, x = 10, was doubtlessly regarded as the ultimate base number in the tribe by most previous workers. In addition, this number has been proposed as the base number for the entire Asteroideae (Robinson et al., 1981). High chromosome numbers, such as n = 15, 16, 17, and 25, have been considered to be the result of polyploid increase followed by dysploid loss (King &

Table 3. Distribution of base chromosome numbers in Eupatorieae according to subtribe (King & Robinson, 1987). Chromosome numbers underlined are confirmed by the present study.

Adenostemmatinae			10							
Eupatoriinae			10							
Disynaphiinae			10							
Gyptidinae			10							
Ageratinae		9	10	11	12					
Ayapaninae			10							
Alomiinae		9	10							
Liatrinae			10							
Fleischmanniinae	4		10							
Critoninae			10							
Praxeiinae			10							
Hebeclinae			10				16			
Trichoroninae						15				
Neomirandeinae								17		25
Mikaniinae								17	18	
Oxylobinae							16	17		
Hofmeisteriinae									18	
Oaxacaniinae									18	

several lineages as shown in Table 1. Polyploidy accompanied by agamospermy is common in the genera *Ageratina, Stevia, Chromolaena, Praxelis* (King & Robinson, 1987), and *Eupatorium* (Watanabe, 1986). The frequent occurrences of polyploids including odd-ploids in these genera are consistent with their previous inferences of widespread agamospermy.

The shrubby or arborescent habit is characteristic of taxa with high chromosome numbers in relatively stable habitats both in Heliantheae and Eupatorieae. Primitive groups of Eupatorieae follow similar evolutionary attributes to some members of Heliantheae. The reduction in chromosome number correlated with the herbaceous habit in Eupatorieae might promote the exploitation of open habitats with ephemeral resources.

Cytology might prove most helpful in subtribal and generic circumscription, and in reflecting some of the evolutionary trends.

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NÚMEROS CROMOSÓMICOS DE ALGUNAS ESPECIES MEXICANAS DE *ARISTIDA* (POACEAE: ARISTIDEAE)[1]

Jorge Sánchez-Ken y Patricia Dávila Aranda[2]

Resumen

Se obtuvieron nuevos registros de números cromosómicos para *Aristida adscensionis* $2n = 22$, *A. divaricata* $2n = 22$, *A. glauca* $n = 22$ y $2n = 44$, *A. laxa* $2n = 44$, *A. schiedeana* $2n = 22$, 44 y *A. ternipes* $2n = 22$. Por primera vez se obtuvo el número cromosómico para *A. curvifolia* y *A. tehuacanensis*. Para *Aristida curvifolia* se registraron los números diploides $2n = 22$ y 44. *Aristida tehuacanensis* presentó un número haploide $n = 11$ y un número diploide $2n = 22$.

Abstract

Chromosome counts are reported as follows: *Aristida adscensionis* $2n = 22$, *A. divaricata* $2n = 22$, *A. glauca* $n = 22$ and $2n = 44$, *A. laxa* $2n = 44$, *A. schiedeana* $2n = 22$, 44, and *A. ternipes* $2n = 22$. The reports of $2n = 22$, 44 chromosome numbers for *A. curvifolia* and $n = 11$ and $2n = 22$ for *A. tehuacanensis* are the first for these species.

El conocimiento citogenético del género *Aristida* dista aún de ser completo, ya que de las aproximadamente 320 especies que se reconocen, sólo para el 20% de ellas se conocen sus números cromosómicos. No obstante, con los datos que se tienen se puede suponer que el número cromosómico básico es $x = 11$ (Gould, 1958; De Winter, 1965; DeLisle, 1969; Allred, 1984).

Por otro lado, del total de números cromosómicos reportados para las especies del género *Aristida*, el 43% representan números poliploides y algunas aneuploidías. El mayor número de poliploides se presenta en las secciones *Aristida* y *Streptachne*, donde el fenómeno de hibridización parece ser un factor común (Henrard, 1929; de Winter, 1965; Allred, 1984) que contribuye a la formación de especies poliploides. En contraste, las aneuploidías se presentan en la secciones restantes del género (de Winter, 1965).

Material y Método

Para llevar a cabo las técnicas citológicas, se obtuvieron semillas de ejemplares herborizados y recolectadas en el campo y se pusieron a germinar en cajas de Petri con algodón húmedo a una temperatura de 30°C.

Cuando las semillas germinaron, se les cortó la raíz primaria y la parte apical de la plúmula donde se encuentra el tejido meristemático. A estas muestras se les aplicó el método rápido de aplastamiento, tiñendo con aceto-orceína para el estudio de cromosomas somáticos, modificado por García (1947). De la misma manera se llevó a cabo la técnica con aceto-carmín para la obtención de cromosomas meióticos, obtenidos de microsporocitos de anteras jóvenes de material fresco fijado en el campo en solución de Farmer.

Todos los vouchers están depositados en el Herbario Nacional de México, (MEXU).

Resultados

En la Tabla 1 se presentan los números cromosómicos obtenidos de las especies de *Aristida* del Valle de Tehuacán-Cuicatlán (Sánchez-Ken, 1991) y de *A. ternipes* de los estados de Yucatán, Guadalajara y Chihuahua. Además, en la Tabla 2 se presentan en forma comparativa los números cromosómicos obtenidos por otros autores.

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Tabla 1. Números cromosómicos registrados para las especies de *Aristida*.

Especie	*n*	2*n*	Localidad
A. adscensionis L.		22	Puebla: Zapotitlán, *Sánchez-Ken et al. 96*
A. curvifolia Fourn.		22	Puebla: Tehuacán, *Sánchez-Ken et al. 330*
		44	Puebla: Tehuacán, *Sánchez-Ken y Tenorio 469*
A. divaricata Humb. & Bonpl ex Willd.		22	Oaxaca: Nochixtlán, *Sánchez-Ken et al. 204*
A. glauca (Nees) Walp.	22		Puebla: Caltepec, *Sánchez-Ken et al. 260*
		44	Puebla: Tehuacán, *Sánchez-Ken et al. 329*
A. laxa Cav.		44	Oaxaca: Huajuapan, *Sánchez-Ken et al. 104*
		44	Puebla: Zapotitlán, *Sánchez-Ken et al. 327*
A. schiedeana Trin. & Rupr.		44	Puebla: Caltepec, *Salinas & Tenorio 5871*
		22	Oaxaca: Coixtlahuaca, *Sánchez-Ken & Tenorio 448*
A. tehuacanensis Sánchez-Ken & Dávila	11		Puebla: Tehuacán, *Sánchez-Ken et al. 331*
		22	Puebla: Tehuacán, *Sánchez-Ken & Tenorio 469*
A. ternipes Cav.		22	Chihuahua, *Blanco 1242*
		22	Jalisco, *Santana 396*
		22	Yucatán, *Medina 91*

Por primera vez se obtuvieron los números cromosómicos para *Aristida curvifolia* y la nueva especie *A. tehuacanensis* (Dávila & Sánchez-Ken, 1994); además se determinaron nuevos registros para *A. adscensionis, A. divaricata, A. glauca, A. laxa, A. schiedeana* y *A. ternipes.*

Para *Aristida curvifolia* se registraron números cromosómicos tanto diploides como tetraploides (2*n* = 22 y 44). Estos números se presentan en *A. glauca, A. laxa* y *A. schiedeana* Gould (1966), Reeder (1971, 1977, 1984), Davidse & Pohl (1972).

Como complemento de los números haploides (*n*) encontrados por Beetle et al. (1983), se presentan los números diploides (2*n*) correspondientes, tanto de *Aristida curvifolia* como de *A. glauca,* las cuales pertenecen al complejo *Purpurea* (Lamson-Scribner, 1901; DeLisle, 1969, 1973; Holmgren & Holmgren, 1977; Allred, 1984).

Con respecto a *Aristida ternipes,* el registro obtenido fue 2*n* = 22 el cual concuerda con lo obtenido por Tateoka (1962) y Beetle et al. (1983). Sin embargo, Gould (1958) obtuvo el registro hap-

Tabla 2. Números cromosómicos registrados para las especies de *Aristida* obtenidos por diversos autores.

Especie	*n*	2*n*	Referencia
A. adscensionis L.		22	Gould, 1966; Gould & Soderstrom, 1970; Davidse & Pohl, 1972; Reeder, 1977; Sánchez-Ken, 1991
	11		Gould, 1960; DeLisle, 1969; Beetle et al., 1983
A. curvifolia Fourn.		22, 44	Sánchez-Ken, 1991
A. divaricata Humb. & Bonpl. ex Willd.	11		Gould, 1958; Beetle et al., 1983; Sánchez-Ken, 1991
		22	Sánchez-Ken, 1991
A. glauca (Nees) Walp.		22	Gould, 1966; Reeder, 1977; Sánchez-Ken, 1991
		44	Reeder, 1977; Sánchez-Ken, 1991
	11, 22		DeLisle, 1969; Beetle et al., 1983
A. laxa Cav.	22		Davidse & Pohl, 1972
		44	Sánchez-Ken, 1991
A. schiedeana Trin. & Rupr.		22	Reeder, 1971, 1984; Sánchez-Ken, 1991
		44	Sánchez-Ken, 1991
A. tehuacanensis Sánchez-Ken & Dávila	11	22	Sánchez-Ken, 1991
A. ternipes Cav.		22	Beetle et al., 1983; Sánchez-Ken, 1991
	22		Gould, 1958

loide $n = 22$, para la misma especie, lo cual indica probablemente una serie poliploide en esta especie. Por otro lado al realizar la revisión de ejemplares, se encontró que el número cromosómico registrado por Tateoka (1962) para *A. ternipes*, en realidad pertenece a *A. floridana*, que es una especie muy cercana a *A. ternipes*.

Otro número registrado fue el de *Aristida laxa* $2n = 44$, que corresponde al registro $n = 22$ encontrado por Davidse & Pohl (1972), demostrando una vez más, que esta especie probablemente presenta una serie poliploide intraespecífica.

Finalmente, los números cromosómicos encontrados para *Aristida adscensionis* y *A. divaricata* (Tabla 1) son similares a los registrados por los autores mencionados en la Tabla 2.

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CHROMOSOME COUNTS OF COMPOSITAE FROM ECUADOR AND VENEZUELA[1]

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Abstract

Forty-three chromosome counts of Compositae are reported from Ecuador and Venezuela. A first generic count is reported for *Stuckertiella* Beauverd (n = 11–12), and first counts also are reported for 15 species in *Ageratina* Spach, *Chromolaena* DC., *Coespeletia* Cuatrec., *Erigeron* L., *Espeletia* Mutis, *Gynoxys* Cass., *Hinterhubera* Sch. Bip., *Oritrophium* (HBK) Cuatrec., *Pentacalia* Cass., *Ruilopezia* Cuatrec., *Senecio* L., *Tanacetum* L., and *Vasquezia* Phil. Additional counts also are provided for 23 populations of previously counted taxa, two of which are new numbers. The taxonomic implications of certain of these counts are discussed.

During research expeditions to Ecuador and Venezuela in the past 15 years, we have collected numerous samples of Compositae. In addition to herbarium material, we have obtained floral buds for chromosomal studies. This paper gives the results of these studies, which are important for helping delimit taxa and reconstructing phylogeny in the family. These investigations extend previous chromosomal work by Keil & Stuessy (1975, 1977), Jansen & Stuessy (1980), and Jansen et al. (1984). This paper lists first counts for genera, species, and varieties, as well as confirmatory data for other taxa known from only one or a few reports. Discussion focuses on taxonomic and/or evolutionary implications.

Materials and Methods

The materials and methods involving conventional squash techniques for meiotic stages of nuclei in pollen parent cells are outlined in Keil & Stuessy (1975, 1977) and Jansen & Stuessy (1980). Either Snow's stain (Snow, 1963) or acetocarmine was used. Voucher specimens of Venezuelan collections are deposited at Instituto Botánico, Venezuela (VEN), and Ecuadorian collections are at Ohio State University (OS).

Results

The 42 new chromosome counts are listed in Table 1. First counts are reported for 1 genus and 17 species, and 23 additional counts are for previously reported taxa, 2 of which are new numbers. The new generic count is for *Stuckertiella* Beauverd. New species counts are in *Ageratina* Spach, *Chromolaena* DC., *Coespeletia* Cuatrec., *Erigeron* L., *Espeletia* Mutis, *Gynoxys* Cass., *Hinterhubera* Sch. Bip., *Oritrophium* (HBK) Cuatrec., *Pentacalia* Cass., *Ruilopezia* Cuatrec., *Senecio* L., *Tanacetum* L., and *Vasquezia* Phil.

Discussion

The discussion here is restricted to first or new counts and to significant comments, which is the approach used in previous papers (Keil & Stuessy, 1975, 1977; Jansen & Stuessy, 1980; Jansen et al., 1984). References for statements regarding ranges of chromosomal variation within genera or for frequently counted species will not be given; documentation for those counts comes from available chromosomal indices (Darlington & Wylie, 1955; Cave, 1958–65; Ornduff, 1967–69; Fedorov, 1969; Moore, 1970–77; Goldblatt, 1981–1988; Goldblatt & Johnson, 1990–1991).

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The referral of genera to particular tribes follows the classification of the Compositae in Heywood, Harborne, and Turner (1977). The sequence of tribes used within subfamilies is alphabetical, but tribal composition of each subfamily follows Bremer (1994), with recognition of tribe Tageteae, after Strother (1977). Chloroplast DNA and morphological cladistic analyses (e.g., Bremer, 1987; Jansen et al., 1992) are in reasonable agreement that the family should be divided into three subfamilies, Barnadesioideae, Asteroideae, and Cichorioideae. Most of the counts reported here come from Asteroideae.

ASTEROIDEAE

Anthemideae. Our count of $n = 12$ for *Tanacetum cinerariaefolium* is very unusual, considering the constancy of $x = 9$ throughout the genus. Heywood & Humphries (1977) also reported several very unusual chromosome numbers for *Tanacetum*, such as $2n = 24$, 34, and 56, but no literature is cited as documentation.

Astereae. *Aster squamatus* has been counted previously as $n = 10$ (Villa, 1978; Jansen & Stuessy, 1980; Dillon & Turner, 1982; Baltisberger, 1988). Our count of $n = 5$ establishes what appears to be the diploid level within this species. Dillon & Turner (1982) distinguished this South American species from the closely related North American taxon, *A. subulatus* Michx., emphasizing differences in characters of the involucre. *Aster subulatus* has repeatedly been found to be diploid ($n = 5$), except for one tetraploid count by Turner (1978) from Florida, and other reports of tetraploidy from Asia and Africa (Turner & Lewis, 1965; Matsuda, 1970; Miyagi, 1974). Our report of a new chromosome level for *A. squamatus*, therefore, suggests that it, too, has undergone infraspecific polyploidy, and that tetraploid cytodemes have evolved independently within each taxon.

Of the 27 species of *Erigeron* found in South America (Solbrig, 1962), 10 species have been counted (*E. andicola* DC., *E. ecuadoriensis* Hieron., *E. fernandezianus* (Colla) Solbrig, *E. karwinskianus* DC., *E. lanceolatus* Wedd., *E. leptopetalus* Phil., *E. leptorhizon* DC., *E. maximus* (D.Don) DC., *E. myosotis* Pers., and *E. rupicola* Phil.). Our count of n = ca. 27 for *E. apiculatus* is the first for the species and is at the hexaploid level. Another first count of $n = 36$–40 for *E. pinnatus* is apparently at the octaploid level. *Erigeron karwinskianus*, a pantropical weed native to Mexico and Central America (Solbrig, 1962), has previously been counted as diploid, triploid ($2n = 27$I), tetraploid, and hexaploid from populations in Mexico and Central America. Our count from South America (Ecuador) is also tetraploid. However, there is also one diploid report from Ecuador for this species (Olsen, 1980). This is the only diploidy reported in the genus *Erigeron* from South America. An interesting observation is that hexaploids and octaploids predominate in South America, whereas diploids largely prevail in North America and Central America. This geographical partitioning between ploidy levels suggests that the genus may have originated in North America, which might be expected based on the greater number and diversity of species in that region.

Only one chromosome count has been reported previously for *Hinterhubera* (*H. imbricata*, $n = 9$; Powell & Cuatrecasas, 1970), which comprises four species in the Andes. Our count of n = ca. 9 for *H. lagesgui* is consistent with the basic number $x = 9$ and is the first count for the species.

Oritrophium, a genus with 15 species mainly distributed in the Andes (Mabberly, 1987) and once treated as a section of *Erigeron* (Bentham & Hooker, 1873), has had, until now, only two chromosomally known species (*O. aciculifolium* Cuatrec., $n = 9$, Turner et al., 1967 and *O. hirtopilosum* (Hieron.) Cuatrec., $n = 18$, Dillon & Turner, 1982). Our new count of n = ca. 17 for *O. venezuelense* is presumably also a tetraploid. The three closely related genera *Aster*, *Conyza*, and *Erigeron* have basic numbers of $x = 9$, which is consistent with the base found in *Oritrophium*.

Eupatorieae. *Ageratina* is composed of five subgenera and about 248 species and has very diverse chromosome numbers (King & Robinson, 1987). Although variations are known to exist within particular species comprising subgenus *Ageratina* (Grashoff et al., 1972; King et al., 1976), the basic number is likely $x = 17$ (King & Robinson, 1987). Among our counts for three species belonging to subgenus *Ageratina*, two, *A. cuencana* (n = ca. 18) and *A. rhypodea* (n = ca. 18), are reported for the first time. *Ageratina azangaroensis*, however, is counted as $n = 12$ and $n = 16$–18 from two different populations from Ecuador. Previously reported chromosome numbers for this species were $n = 17$, with or without one or more fragments (King et al., 1976). Whether our count of $n = 12$ reflects an aberrant plant, a stabilized cytotype, or apomixis cannot be determined at this time. More sampling is needed.

Chromolaena has over 165 known species (King & Robinson, 1987); only 17 species have been counted (King & Robinson, 1970; King et al.,

Table 1. Chromosome counts of Compositae from Ecuador and Venezuela. First counts designated by **; new chromosome level by *.

Taxon	Gametic chromosome number[a]	Voucher[b]
ASTEROIDEAE		
ANTHEMIDEAE		
Chrysanthemum parthenium (L.) Bernh.	ca. 9	ECUADOR. Chimborazo: 6.8 km NE of Cajabamba, *SN 5825.*
Cotula australis (Less.) Hook. f.	9	ECUADOR. Loja: 50.6 km N of Loja, *SN 5909.*
***Tanacetum cinerariaefolium* Sch. Bip.	12	ECUADOR. Tungurahua: 21.9 km N of Riobamba, *SN 5941.*
ASTEREAE		
**Aster squamatus* (Spreng.) Hieron.	5	ECUADOR. Cañar: 7.7 km N of Cañar *SN 5787.*
Baccharis latifolia (Ruiz & Pav.) Pers.	18	ECUADOR. Pichincha: 2.2 km N of Tambillo, *SN 5787.*
Blakiella bartsiifolia (S. F. Blake) Cuatrec.	9	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3959*
***Erigeron apiculatus* Benth.	ca. 27	ECUADOR. Tungurahua: 21.9 km N of Riobamba, *SN 5943.*
Erigeron karwinskianus DC.	ca. 18	ECUADOR. Azuay: 20.9 km S of Cuenca, *SN 5925.*
***Erigeron pinnatus* Turcz.	36–40	ECUADOR. Chimborazo: 6.8 km NE of Cajabamba, *SN 5824.*
Hinterhubera imbricata Cuatrec. & Aristeg.	9	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3886.*
	ca. 10	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3954.*
***Hinterhubera lagesgui* Wedd.	ca. 9	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3953.*
***Oritrophium venezuelense* (Steyerm.) Cuatrec.	ca. 17	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3873.*
EUPATORIEAE		
Ageratina azangaroensis (Sch. Bip. ex Wedd.) R. M. King & H. Rob.	12	ECUADOR. Chimborazo: 15.0 km SW of Cajabamba, *SN 5837.*
	16–18	ECUADOR. Cotopaxi: 16.2 km S of Machachi, *SN 5952.*
***Ageratina cuencana* (B. L. Rob.) R. M. King & H. Rob.	ca. 18	ECUADOR. Tungurahua: along path from W end of Baños to Chaupi, *SN 5800.*
Ageratina exerto-venosa (Klatt) R. M. King & H. Rob.	36–40	ECUADOR. Loja: 21.4 km NNW of Saraguro, *SN 5921.*
***Ageratina rhypodea* (B. L. Rob.) R. M. King & H. Rob.	ca. 18	ECUADOR. Loja: 13.9 km NE of Saraguro, *SN 5916*
***Chromolaena leptocephala* (DC.) R. M. King & H. Rob.	9	ECUADOR. Tungurahua: on path from W end of Baños to Chaupi, *SN 5804.*
Oxylobus glanduliferus (Sch. Bip.) A. Gray	16	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3879.*
HELIANTHEAE		
Ambrosia peruviana Willd.	18	ECUADOR. El Oro: 1.8 km N of El Cambio, *SN 5859.*
***Coespeletia timotensis* (Cuatrec.) Cuatrec.	19	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3966.*
	19	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3883.*
***Espeletia batata* Cuatrec.	19	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3874.*

Table 1. Continued.

Taxon	Gametic chromosome number[a]	Voucher[b]
Garcilassa rivularis Poepp. & Endl.	ca. 16	ECUADOR. Chimborazo: NW edge of Bucay, *SN 5854.*
Monactis flaverioides H.B.K.	30–32	ECUADOR. Pichincha: 2.2 km N of Tambillo, *SN 5786.*
	ca. 30	ECUADOR. Tungurahua: 38.4 km N of center of Riobamba, *SN 5945.*
***Ruilopezia floccosa* (Standl.) Cuatrec.	19	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3965.*
Sigesbeckia jorullensis H.B.K.	ca. 16	ECUADOR. Loja: 50.6 km N of Loja, *SN 5911.*
Steiractina mollis S. F. Blake	14	ECUADOR. Cañar: 6.7 km S of Suscal, *SN 5931.*
***Vasquezia titicacensis* (Meyen & Walp.) S. F. Blake	20	ECUADOR. Cañar: 3.3 km N of Cañar, *SN 5927.*
INULEAE		
Gamochaeta americana (Mill.) Wedd.	14	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3920.*
***Stuckertiella capitata* (Wedd.) Beauverd	11–12	ECUADOR. Chimborazo: 10.5 km S of Cajabamba, *SN 5830.*
SENECIONEAE		
***Gynoxys buxifolia* Cass.	ca. 36	ECUADOR. Chimborazo: 15.0 km SW of Cajabamba, *SN 5838.*
	ca. 12	ECUADOR. Cotopaxi: 12.6 km S of Machachi, *SN 5951.*
***Pentacalia sclerosa* (Cuatrec.) Cuatrec.	ca. 20	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3906.*
Senecio formosus H.B.K.	40	VENEZUELA. Mérida: Páramo Piedras Blancas, *BG 3870.*
***Senecio teretifolius* (H.B.K.) DC.	20	ECUADOR. Chimborazo: 8.6 km NW of center of Riobamba, *SN 5938.*
TAGETEAE		
Tagetes zypaquirensis Humb. & Bonpl.	ca. 12	ECUADOR. Chimborazo: 9.7 km NW of center of Riobamba, *SN 5940.*
CICHORIOIDEAE		
LIABEAE		
Liabum floribundum Less.	18	ECUADOR. Chimborazo: 44.4 km SW of Cajabamba, *SN 5845.*
**Erato sodiroi* (Hieron.) H. Rob.	9	ECUADOR. Chimborazo: 34.1 km N of jct. rd. to Huigra, *SN 5934.*
MUTISIEAE		
Onoseris albicans (D. Don) Ferreyra	18	ECUADOR. Azuay: 54.2 km NNE of Saraguro, *SN 5923.*
Perezia multiflora (Humb. & Bonpl.) Less.	8	ECUADOR. Chimborazo: 10.5 km S of Cajabamba, *SN 5827.*

[a] Numbers represent bivalents.
[b] Ecuadorian collections by *Stuessy & Nesom* (*SN*); Venezuelan collections by *Berry & Gómez* (*BG*).

1976; Mathew & Mathew, 1983; Strother, 1983; Baker & Parfitt, 1986; Bernardello, 1986; Sundberg et al., 1986). Most previous reports suggest that the basic number of this genus is $x = 10$, although some variations have been found due to irregular meiosis (for *C. laevigata* (Lam.) R. M. King & H. Rob., $2n =$ ca. 56I, Sundberg et al., 1986; for *C. odorata* (L.) R. M. King & H. Rob., $2n = 58$, Ghosh, 1961, $n =$ ca. $31 + 4$I, King et al., 1976). Our count of $n = 9$ with clear bivalents

for *C. leptocephala* suggests that it is an aneuploid within the genus.

Heliantheae. *Coespeletia* (Espeletiinae), with eight species once included in *Espeletia* (Smith & Koch, 1935) but separated by Cuatrecasas (1976), has two chromosomally known species with $n = 19$ (*C. moritziana* (Sch.Bip. ex Wedd.) Cuatrec., *C. thyrsiformis* (Smith) Cuatrec.; Powell & Cuatrecasas, 1975). Our new counts of $n = 19$ for two individuals of *C. timotensis* (Cuatrec.) Cuatrec. are consistent with these reports. Another generic segregate, *Ruilopezia*, is known to have $n = 19$ in *R. bromelioides* (Cuatrec.) Cuatrec. (Powell & Powell, 1978) and $n = 19$ in a possible intergeneric hybrid between *R. floccosa* and *Espeletiopsis pannosa* (Standley) Cuatrec. (Powell & Cuatrecasas 1970; cited as *Espeletia floccosa* × *Espeletia pannosa*). Our count of $n = 19$ is the first for *R. floccosa*. In addition, *Espeletia batata* has been newly counted as $n = 19$. The counts for these species are consistent with the rest of Espeletiinae, which contains seven genera that include species previously placed in *Espeletia* Mutis ex Humb. & Bonpl. (Cuatrecasas, 1976). More than 50 species have been counted, all as $n = 19$ (Powell & King, 1969; Powell & Cuatrecasas, 1970, 1975). Despite the obvious morphological differences among these newly created genera, they appear to represent a monophyletic unit. The putative hybrid between two of these genera underscores their close affinity, as does the uniform chromosome number for all species of the subtribe counted to date. A more taxonomically meaningful approach might be to recognize sections and subgenera within a diverse single genus, *Espeletia*.

Our new count of $n = 20$ for *Vasquezia titicacensis* is consistent with previous reports (*V. achillioides* (Less.) Less., $n = 9$, Olsen, 1980; *V. anemonifolia* (HBK) S. F. Blake, $n = 19$, Powell & King, 1969; Jansen et al., 1984; *V. oppositifolia* (Lag.) S. F. Blake, $n = 20$, Dillon & Turner, 1982). Dillon & Turner (1982) suggested that this genus has a basic number of $x = 9$ or 10, in which case our report for *V. titicacensis* is also at the tetraploid level.

Inuleae. *Stuckertiella* is a genus with only two known species, both in S. America: *S. capitata* and *S. peregrina* Beauverd (Beauverd, 1913; Merxmüller et al., 1977). Our count of $n = 11$–12 for *S. capitata* is the first report for this genus. *Gamochaeta* is the closest generic relative, itself sometimes treated as a section of *Gnaphalium* (Beauverd, 1913). The basic chromosome number of *Gnaphalium* sensu lato is suggested as $x = 7$, a number which, along with $x = 9$ and 10, is predominant in Inuleae (Merxmüller et al., 1977). Our count might be interpreted as coming from a descending aneuploid from $n = 14$, a number also occurring in the tribe (Merxmüller et al., 1977).

Senecioneae. *Gynoxys*, with about 100 species, has only three chromosomally known taxa (*G. fuliginosa* (HBK) Cass., $n =$ ca. 40, Hunziker et al., 1989; *G. parvifolia* Cuatrec., $n =$ ca. 40; and *G. tomentosissima* Cuatrec., $n = 40$ + fragment, Turner et al., 1967). The basic number of this genus has been suggested to be $x = 10$ by Turner et al. (1967). Our new counts from two different populations in Ecuador for *G. buxifolia* are $n =$ ca. 12 and $n =$ ca. 36, which could indicate a basic number of $x = 12$, or perhaps dibasic with $x = 10$ and $x = 12$.

Our counts for *Senecio*, one of which is reported here for the first time (*S. teretifolius*), are consistent with the previously established basic number $x = 10$, although Turner and Lewis (1965) report three African species with $2n = 10$ ($x = 5$). Our count of $n =$ ca. 20 for *Pentacalia sclerosa* is consistent with previous numbers based on $x = 10$.

CICHORIOIDEAE

Liabeae. Our count of $n = 9$ for *Erato sodiroi* is a new level for the species, which has been previously counted as $n = 11$ (Strother and Panero, 1994). While our count and that of Olsen (1980) for *E. polymnioides* DC. support the suggestion by Robinson et al. (1985) that the basic number of this genus is $x = 9$, additional reports for *E. polymnioides* of $n =$ ca. 11 (Sundberg & Dillon, 1986) from material taken in Huánuco, Peru, and $2n =$ ca. 20 with possible multivalents from Ecuadorian material (Strother & Panero, 1994) could point to more complex chromosomal patterns within the genus.

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WOOD AND BARK ANATOMY OF *NOTHOTSUGA* (PINACEAE)[1]

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ABSTRACT

Nothotsuga longibracteata Hu ex Page is one of the rare gymnosperms endemic to China. Although previously ascribed to the genus *Tsuga*, it was recently segregated as a monotypic genus on account of its distinctive exomorphology. The wood and bark structure described here show that it has unique characteristics as well as features in common with *Tsuga*. The presence of vertical normal resin canals in the wood, together with resin cavities in the bark, does not support the earlier assignment of *Nothotsuga* to the section *Heopeuce* Keng & Keng f. of *Tsuga*, nor does it fit any other pinaceous taxon. Thus the present study supports the view that *Nothotsuga* should be validated as a genus in Pinaceae.

Nothotsuga Hu ex C. N. Page is a monotypic genus and one of the rare gymnosperms endemic to China (Page, 1988). It is an evergreen forest tree up to about 30 m high, distributed in a few restricted areas of Guizhou, Hunan, Guangdong, and Fujian provinces of China (Fig. 1). With changes in the environment and ecosystems, the total number of individuals has decreased rapidly, and it is considered an endangered plant on the brink of extinction (Fu & Jin, 1992).

Nothotsuga was first described by Cheng (1932) as a new species in *Tsuga*. Hu (1951) placed it in a separate genus, *Nothotsuga*, on the basis of its arrangement of male strobili and the position and morphology of the female cones, which differ markedly from those in *Tsuga*. Since then, several views about its systematic position have been expressed. Gaussen (1966) treated it as a generic intermediate between *Tsuga* and *Keteleeria*, citing *Tsuga chinensis* (Franch.) Pritzel and *Keteleeria evelyniana* Masters as its likely parents. Cheng & Fu (1978) considered it to be a section under *Tsuga*. Recently, Page (1988) supplied the Latin description of the genus *Nothotsuga* to validate Hu's publication of the genus as a nomen nudum and made additional comments about the differences between *Nothotsuga* and *Tsuga*. Nevertheless, controversy still remains, since little evidence from comparative studies is available.

In this paper, the wood and bark structures of *Nothotsuga* are described in detail based on observations of both light (LM) and scanning electron microscopy (SEM), and a comparison between *Nothotsuga* and other related taxa is made in order to evaluate the systematic position and phylogenetic relationships of *Nothotsuga* in Pinaceae.

MATERIALS AND METHODS

Wood and bark samples were obtained from two different trees grown in the subtropical region of China. The first was taken from the trunk of a living tree occurring naturally in Liancheng county of Fujian province. This sample was immediately fixed in FAA. The second, an air-dried sample from a wood disc, was obtained from the wood collection of Fujian Forestry College.

Wood samples were boiled in water and then sectioned on a sliding microtome. Bark materials were sectioned after penetration with polyethylene glycol. All sections were stained with safranin and mounted in Canada balsam. Macerations were made according to Franklin's (1945) method and mounted in glycerin jelly. Quantitative data were based on 30 measurements per sample. For SEM analysis, small blocks about 1 to 5 mm in thickness were cut off from both wood and bark samples. The transverse and radial surfaces to be observed were cleanly cut with a new razor blade. They were then soaked in a 20% solution of sodium hypochlorite until the surface lost color (Exley et al., 1977), followed by washing in running water. After dehydration through a graded alcohol series, they were mounted on stubs and coated with carbon and gold in a high vacuum evaporation unit. They were examined at 20 kV under a scanning electron microscope.

WOOD DESCRIPTION

Growth rings are distinct with abrupt transition from earlywood to latewood. Earlywood tracheids range from 24 to 37 μm in tangential diameter and from 0.87 to 3.6 mm in length. Tracheid cross-sec-

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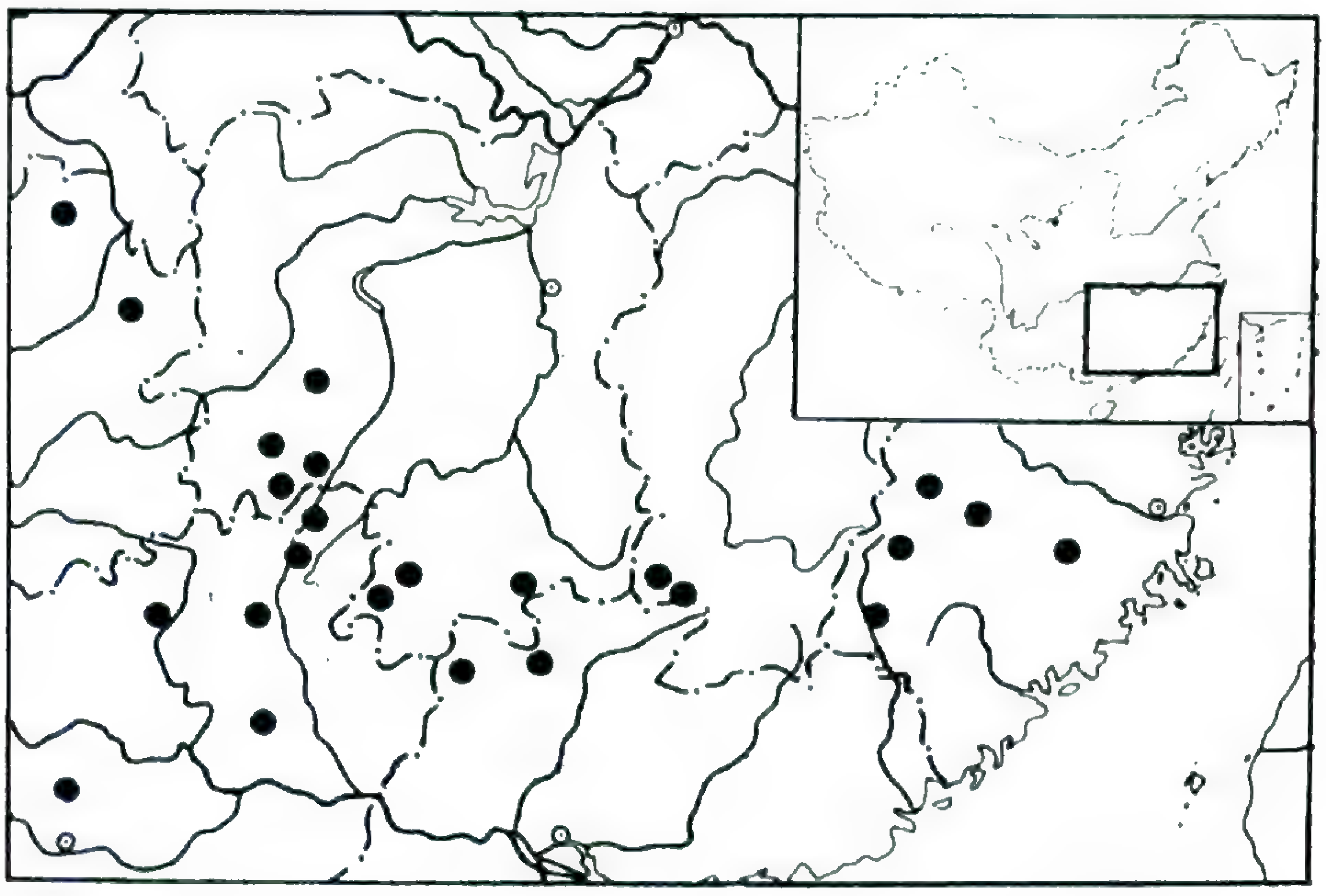


FIGURE 1. Map showing the geographical distribution of *Nothotsuga* in China (courtesy of Fu & Jin, 1992).

tional outlines are usually rounded and sometimes angular. Bordered pits are 18 (16–19) μm in diameter, almost exclusively uniseriate or occasionally irregular in the wider earlywood tracheids. Pit membranes have a centrally thickened torus with a more or less smooth edge. The pit membranes have thickened bars radiating from the torus. Pit apertures are rounded in earlywood, lenticular in latewood with axial or radial orientation. Tracheids have a warty layer lining the inner surface of the secondary wall and in the pit chambers. Trabeculae are absent.

Axial parenchyma are scarce and diffuse, arranged on the margin of the growth rings with a tendency to form a discontinuous line. Cross-sectional outlines are angular, nearly isodiametric in earlywood, radially flattened in latewood. The end walls of the cells are pitted and mostly nodular. Individual axial parenchyma cells are 162 (112–263) μm in length, 29 (22–34) μm in tangential diameter. Cells are commonly filled with dark-colored contents.

Normal and traumatic vertical resin canals are present. Normal resin canals are elongated and usually occur singly or in a pair in the boundary of latewood (Fig. 2). The number of normal resin canals is extraordinarily low in the juvenile region, but increases with age in the mature region. The size of normal resin canals also changes significantly with age, the oldest canals being the largest. The epithelial cells of normal resin canals are thick-walled, with six cells (5–9) per canal. Traumatic resin canals are characteristically cystlike and form short tangential series or are solitary in the boundary between earlywood and latewood (Figs. 3, 4). Their epithelial cells are also thick-walled, but they are distinguishable on account of their large size and large number of epithelial cells. Transverse resin canals are not observed.

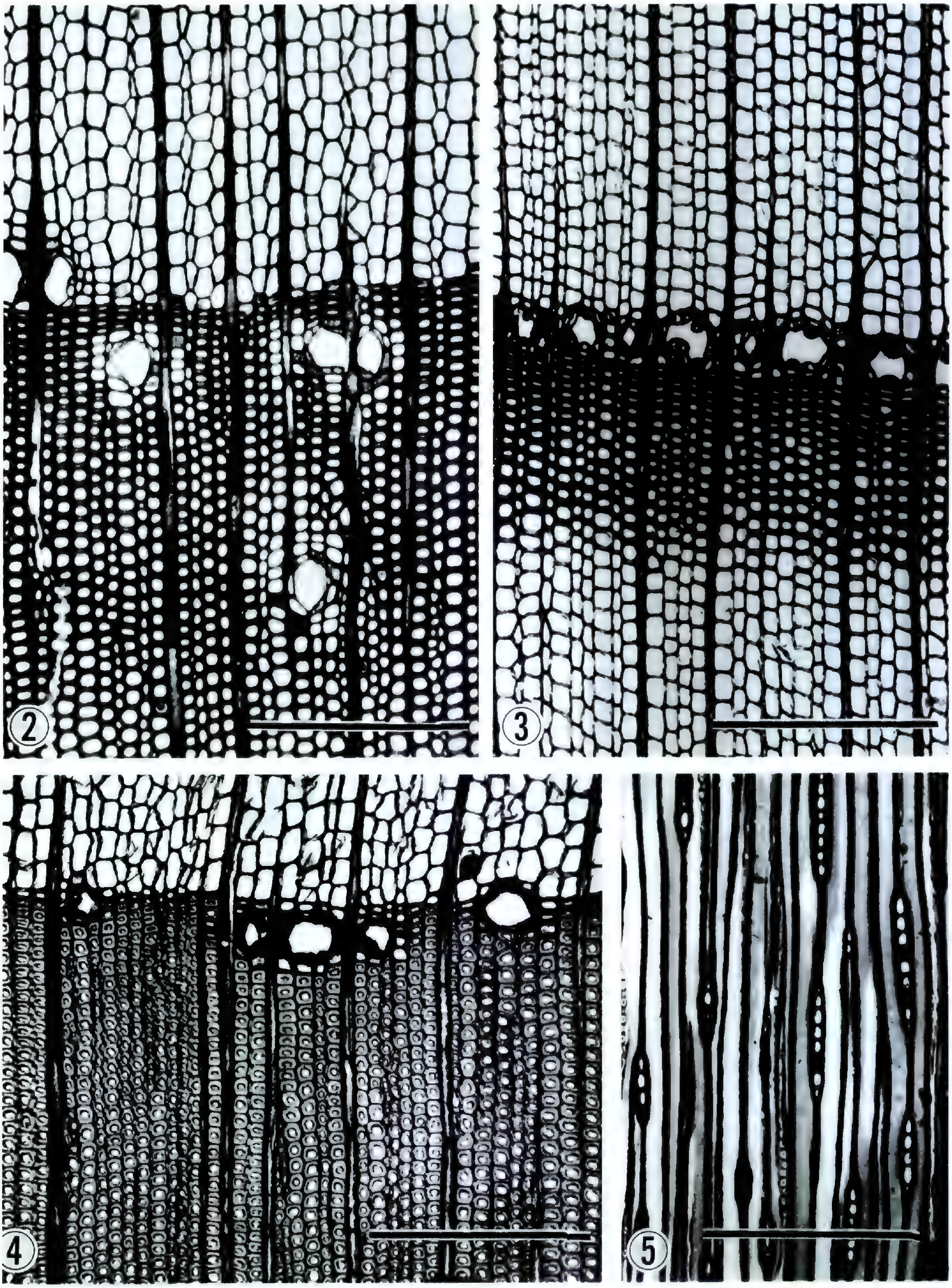
Rays are uniseriate, rarely biseriate, 23 (2–35) cells high, 31 rays per mm^2 in tangential section (Fig. 5). Rays are composed of ray parenchyma and poorly developed ray tracheids (Fig. 6); the latter only occur in the margin rows of the ray. The horizontal walls of ray parenchyma are thick and strongly pitted. The end walls of ray parenchyma cells are thin without indentures or occasionally with small nodules (Fig. 7). Cross-field pits are taxodioid and cupressoid, with two or four pits per cross field (Fig. 8). Square or rectangular crystals are visible in lumina of the ray parenchyma.

BARK DESCRIPTION

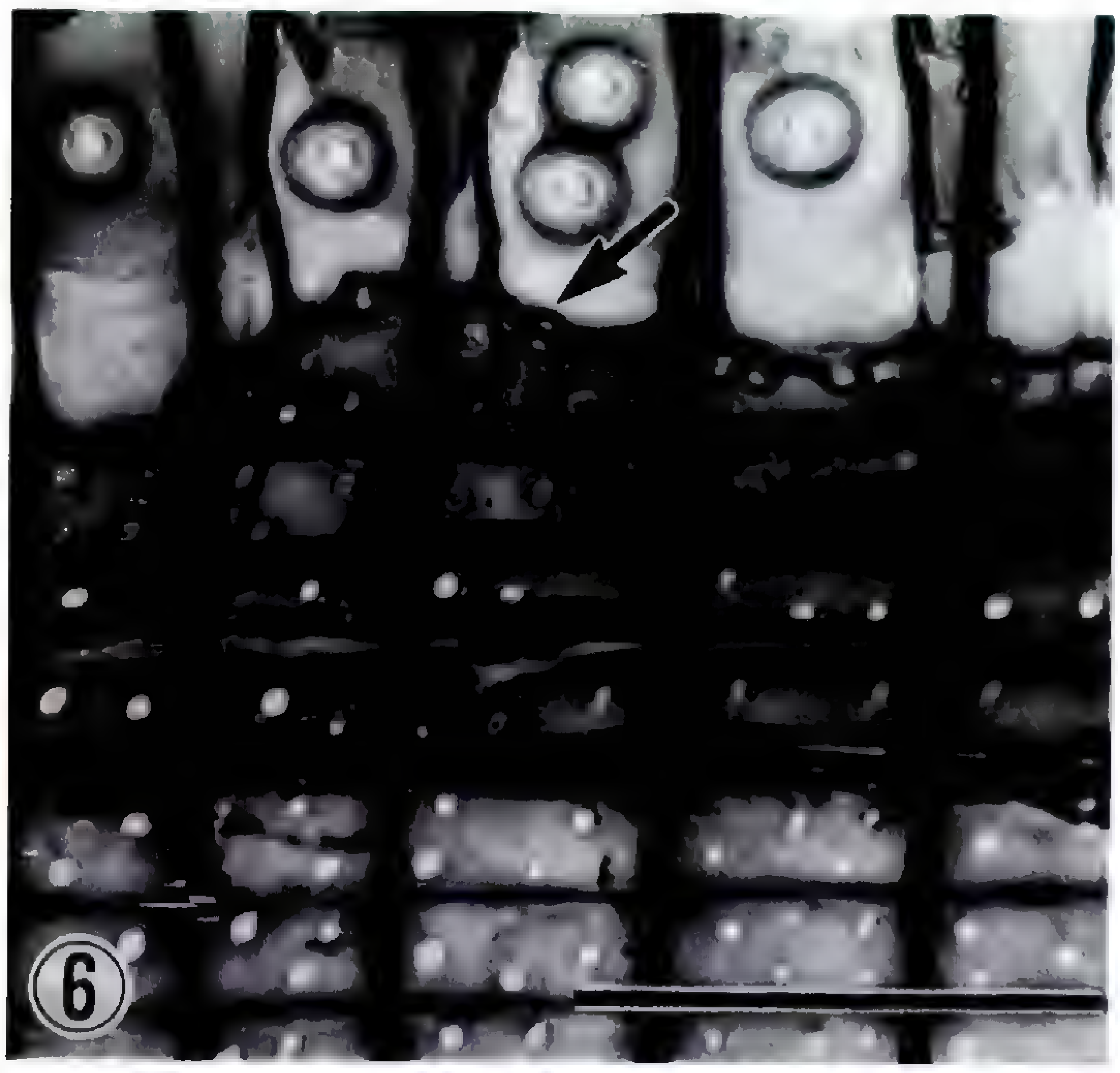
The phloem consists of sieve cells, axial parenchyma, ray parenchyma, resin cavities, and sclereids. Sieve cells and axial parenchyma appear randomly arranged, while sclereids occur in irregular radial rows.

Sieve cells appear rectangular in the transverse section of the functional zone; however, they are crushed by sclereids and rays in the nonfunctional zone. Sieve areas are 8 (3–12) μm diam., mostly in the radial walls in single files.

Axial parenchyma cells are rectangular to isodiametric in transverse section and rectangular in longitudinal section. They are mostly filled with tannin. Square or rectangular crystals are located in some parenchyma cells but are sometimes absent in the non-functional phloem.



FIGURES 2–4. Transverse sections of wood.—2. Normal resin canals in the mature portion of wood.—3. Traumatic resin canals forming a tangential series in the juvenile portion of wood.—4. Solitary and tangential arrangement of traumatic resin canals in the mature portion of wood. FIGURE 5. Tangential section of mature wood. Scale bars in 2, 3, 4 and 5 = 20 μm.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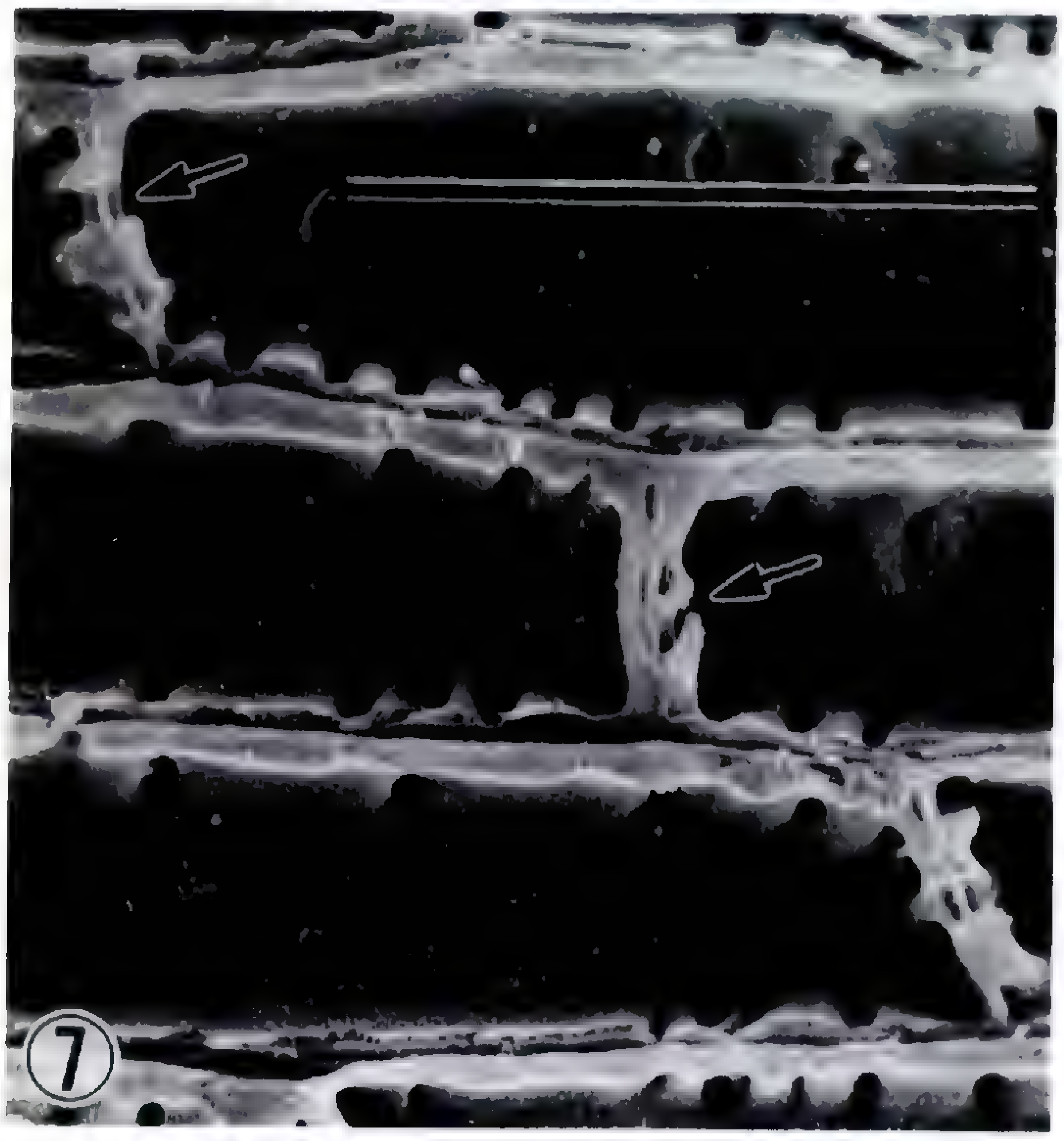
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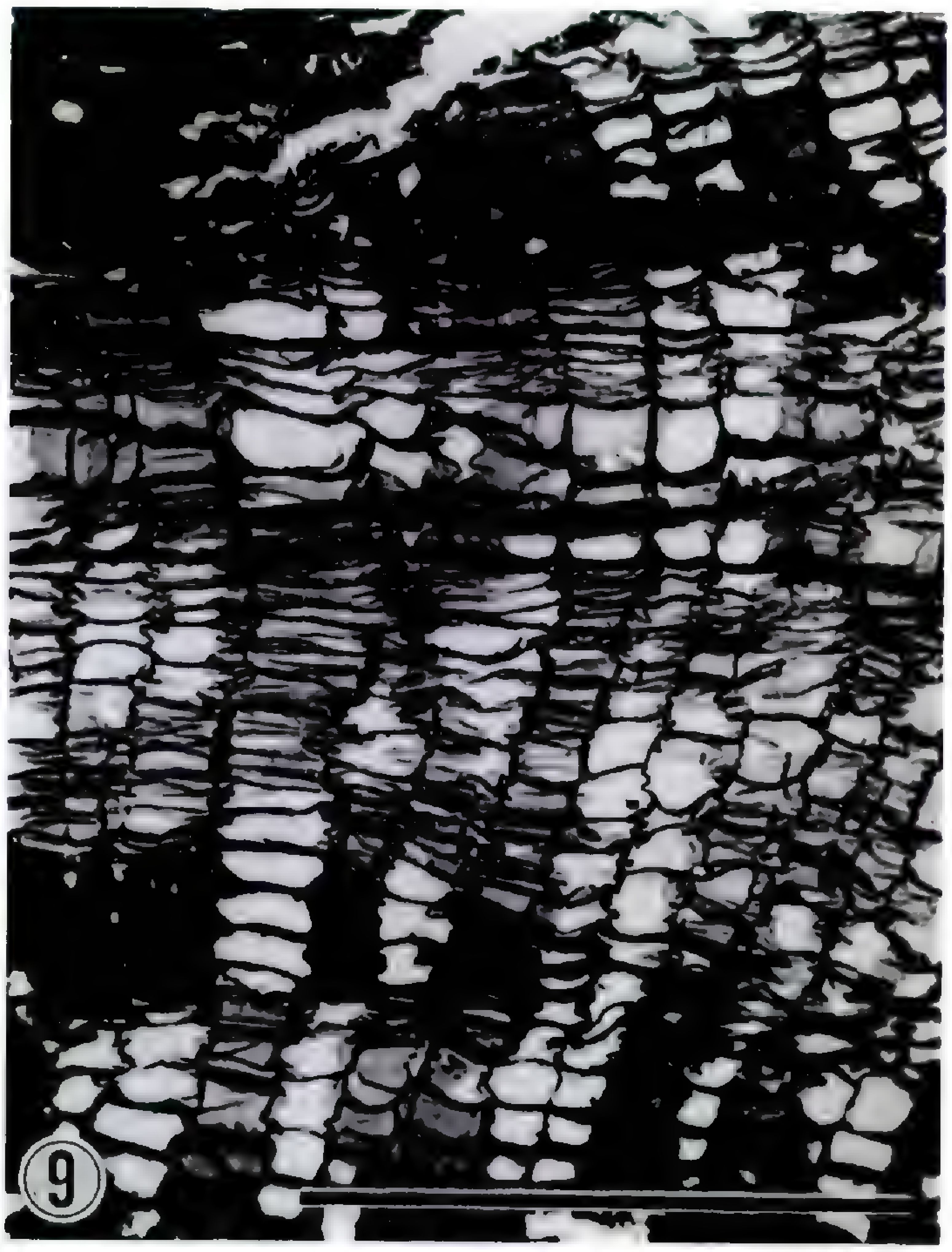
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Rc
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Sc
10

Phloem rays are usually uniseriate, although not infrequently some biseriate rays occur. In height they are 22 (2–28) cells or approximately 700 μm. There are about 23 (12–26) rays per mm^2 tangentially as measured on the cross section; short rows of erect marginal cells are occasionally found in a few of the phloem rays.

Resin cavities are seen in the outer part of inner bark (Fig. 9). They occur singly or in groups of two or three and are abundant in old bark. Some of the larger cavities are found to 120–150 μm diam. and 150–180 μm in axial extent, whereas some of the cavities lack definite borders surrounding them. In prepared slides, they are often filled with a whitish slimy substance.

Sclereids are roundish or polygonal with very narrow lumina in cross section and irregular in longitudinal section. They are invariably filled with brown tannins or resin substances. The walls are lignified, birefringent, and seem to be made up of polylamellate layers. In young twigs sclereids are distributed singly or in masses of 2–4 cells, while they are groups of 5–8 aggregated in "cigar-shaped" or fusiform profiles in old stems.

The periderm consists of 2–3 layers of phelloderm, one layer of phellogen, and alternate layers of thin-walled phellem cells and thick-walled cells, which are probably originated from phelloderm (Fig. 10). Both types of phellem cells show similar shape in outline. They are 14 (12–18) μm and 22 (20–25) μm in radial and tangential dimensions, respectively. The thick-walled cells are characterized by narrow lumina and distinct simple pits, while the thin-walled cells are rather uniform in thickness and with large lumina. Both types of cells contain a resinous substance. Phelloderm cells are arranged in definite radial rows, rectangular to isodiametric in transverse section and radial section. They are sometimes merged into parenchyma cells of secondary phloem tissues.

Discussion

As noticed by Page (1988), this genus occupies a position linking with several other genera of Pinaceae. For example, the shoot and pulvinus form, twig layout, number of resin canals, a sunken midrib in the leaves, and pendulous leading shoots are close to those of *Tsuga* (Yao & Hu, 1982; Page, 1988), while its female cones more closely resemble *Keteleeria* or *Abies* in erect habit, *Pseudotsuga* in size, shape, structure, and number of scales (Napp-Zinn & Hu, 1989; Hu et al., 1989), and *Larix* in exserted, simple bracts and in long, leafy cone peduncles (Farjon, 1990). Further, the unusual grouping of the male cones into clusters appears to link it also with *Pseudolarix* and, more distantly, perhaps with *Cathaya* (Hu et al., 1976; Hu & Wang, 1984; Page, 1988). Because most of its characteristic features are intermediate between *Keteleeria* and *Tsuga* (Gaussen, 1966), it has been considered by van Campo-Duplan & Gausen (1948) to be a recent hybrid between the two genera. Based on fossil evidence of *Palaeotsuga,* plants of probable nearest affinity with *Tsuga longibracteata* occurred in the Pliocene of both Japan and Russia (Miki, 1954; Karavaev, 1958). Page (1988) and Frankis (1988) argued that it was not a modern hybrid.

In studying the wood structure of *Tsuga-Keteleeria longibracteata,* Yu (1956) found no normal vertical resin canals and no important structural diversities between *Tsuga-Keteleeria* and *Tsuga.* Thus, he concluded that there was no necessity of raising the species to the rank of genus. His point of view was also accepted by Cheng & Fu (1978) in compiling the *Flora Reipublicae Popularis Sinicae.*

In the present study, we notice that there are normal resin canals in the wood. In the juvenile portion, few normal resin canals are sporadically observed and they might be missed where scarce. However, there is an increasing tendency towards more resin canals in mature wood, and they can readily be seen in the wood older than 30 years. We are uncertain why Yu (1956) found no normal vertical resin canals in the wood of *Tsuga-Keteleeria longibracteata.* He did not mention where the tree came from nor how old the tree was in his study, but we presume that he was looking for normal resin canals in the juvenile wood rather than in the mature wood.

At wounds, traumatic resin canals in *Nothotsuga* appear to be of sporadic distribution in addition to tangential series. Similar distribution patterns were

←

FIGURES 6–8. Radial sections of wood.—6. Poorly developed ray tracheid (arrow).—7. SEM, Showing ray parenchyma with strongly pitted horizontal walls and nodular end walls (arrows).—8. SEM, showing taxodioid pitting in the cross-field (arrows). FIGURE 9. Cross section of inner bark. FIGURE 10. Cross section of outer bark, showing alternating arrangement of thin- and thick-walled phellem cells. Sc = Sclereids, Rc = Resin cavity. Scale bars in 6, 7 = 5 μm; in 8 = 1 μm; in 9 = 20 μm; in 10 = 30 μm.

Table 1. Differences in wood and bark features among *Nothotsuga*, *Keteleeria*, and *Tsuga*. TGR: Transition of growth rings; NVRC: Normal vertical resin canals; Rc: resin cavities.

Features	*Keteleeria*	*Nothotsuga*	*Tsuga*
1. TGR	gradual	abrupt	abrupt
2. Ray tracheids	none	poorly developed	fully developed
3. Cross-field	taxodioid	taxodioid, cupressoid	cupressoid
4. NVRC	present	present	absent
5. Rc	present	present	absent

observed in *Keteleeria* by Bannan (1936), who studied the conifers quite extensively. He believed that the scattered distribution in *Keteleeria* differed from *Tsuga* and *Abies*, where the associated canals were almost invariably in tangential series. Although the relationship of the scattered canals to injury has not been determined, in this respect *Nothotsuga* resembles such genera as *Keteleeria*, *Larix*, *Pseudotsuga*, and *Picea*.

Resin cavities in the bark are believed to arise through modification of large-sized marginal ray cells or phloem parenchyma cells without further differentiation of border cells. Similar structure has been reported in *Abies* (Srivastava, 1963), *Larix*, *Picea*, *Pinus*, and *Pseudotsuga*, but not in *Tsuga* (Chang, 1954; Lotova, 1975).

The comparisons in Table 1 suggest that several structural features of *Nothotsuga* do not correspond well with any of the existing pinaceous taxa. In particular, a comparison with *Keteleeria*, one of the genera often considered a possible relative, illustrates the differences: the transition from earlywood to latewood in *Keteleeria* is gradual in contrast to the abrupt transition in *Nothotsuga*. Additionally, *Keteleeria* lacks ray tracheids (Greguss, 1955; Phillips, 1963) while *Nothotsuga* possesses ray tracheids. When comparing the structure of *Nothotsuga* with that of *Tsuga*, another genus most often assumed having close affinities, we also found some features that are not in agreement. In addition to possession of normal resin canals in the wood and resin cavities in the bark mentioned above, *Nothotsuga* is characterized by poorly developed ray tracheids occasionally on either side of rays, which differ from the fully developed ray tracheids in *Tsuga*. Furthermore, the cross-field pitting in *Nothotsuga* is mainly taxodioid, occasionally cupressoid or piceoid, while it is mainly cupressoid in *Tsuga* as noticed by Cheng (1980) and Ho et al. (1984).

The wood and bark structure described above indicates that *Nothotsuga* does not fit well in either *Tsuga* or *Keteleeria*. The presence of normal vertical resin canals in the wood, together with resin cavities in the bark of *Nothotsuga*, weighs heavily against some of the earlier treatments where it was included within the genus *Tsuga*, for example, in the section *Heopeuce* Keng & Keng f. of *Tsuga* (Cheng & Fu, 1978). Some characters, particularly the occurrence of ray tracheid in the wood, also exclude *Nothotsuga* from the genus of *Keteleeria*. In conclusion, we support the view that *Nothotsuga* should be validated as a separate genus in the Pinaceae and put between *Keteleeria* and *Tsuga*.

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